

COMPARISON OF MASSETER MUSCLE CHANGES IN TOBACCO CHEWERS AND NON-CHEWERS USING ULTRASONOGRAPHY

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In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY



BRANCH IX

ORAL MEDICINE AND RADIOLOGY

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CERTIFICATE

This is to certify that this dissertation titled "**COMPARISON OF MASSETER MUSCLE CHANGES IN TOBACCO CHEWERS AND NON- CHEWERS USING ULTRASONOGRAPHY**" is a bonafide record of work done by **Dr. Priya. R** under my guidance during her postgraduate study period **2010-2013**.

This dissertation is submitted to **THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY**, in partial fulfillment for the degree of **MASTER OF DENTAL SURGERY, BRANCH IX – Oral Medicine & Radiology**.

It has not been submitted (partial or full) for the award of any other degree or diploma.

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LIST OF ABBREVIATIONS

S.NO	ABBREVIATION	EXPANSION
1.	Akt	Ak thymoma
2.	ALARA	As low as reasonably achievable
3.	ATP	Adenosine triphosphate
4.	B mode	Brightness mode
5.	BTFA	Branch of transverse facial artery
6.	CSA	Cross sectional area
7.	DHS	Demographic and health survey
8.	ECA	External carotid artery
9.	EMG	Electromyography
10.	FA	Facial artery
11.	FFT	Fast fourier transformation
12.	FGF	Fibroblast growth factor
13.	FH	Frankfort horizontal
14.	FNAB	Fine needle aspiration biopsy
15.	FNAC	Fine needle aspiration cytology
16.	GABA	Gamma amino butyric acid
17.	GE	General electric
18.	GH	Growth hormone
19.	IGF-1	Insulin derived growth factor 1
20.	IL 8	Interleukin 8
21.	KHz	kilohertz

22.	MCP	Monocyte chemoattractant protein
23.	MHz	Mega Hertz
24.	MIP	Macrophage inflammatory protein
25.	mm	millimeter
26.	MMH	Masseter muscle hypertrophy
27.	MRI	Magnetic resonance imaging
28.	Msa	Masseteric artery
29.	MSTP	Manufactured smokeless tobacco product
30.	MTOR	Mammalian target of rapamycin
31.	Mxa	Maxillary artery
32.	MyD	Myotonic dystrophy
33.	NCD	Non communicable diseases
34.	Pax	Paired box protein
35.	PDK	Phosphodependant kinases
36.	PI3K	Phosphatidylinositol tri kinase
37.	PKB	Phosphokinase B
38.	PPS	Probability proportional size
39.	PTEN	Phosphatase and tensin analog
40.	SD	Standard deviation
41.	SEAR	South east Asian region
42.	SPL	Spatial pulse length
43.	TGF	Transforming growth factor
44.	TGF- β	Transforming growth factor beta
45.	TMJ	Temporomandibular joint

46.	USA	United states of America
47.	US	Ultrasound
48.	VAS	Visual analog scale
49.	VEGF	Vascular derived endothelial growth factor
50.	WHO	World health organization

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ABSTRACT

STUDY TITLE: COMPARISON OF MASSETER MUSCLE CHANGES IN TOBACCO CHEWERS AND NON CHEWERS USING ULTRASONOGRAPHY

Background and Objectives: The introduction of commercial pan masala, dehydrated and non-perishable powdered areca nut, slaked lime, catechu and cardamom with or without tobacco available in attractive sachets has enhanced the use of smokeless tobacco in India leading to detrimental health effects. Frequent and prolonged chewing of tobacco exerts pressure on muscles of mastication which may result in hypertrophy of muscle. The aim of the study was to measure thickness of masseter muscle at rest and at contraction in subjects with tobacco chewing habit and in control group by ultrasonography, and also to determine blood flow by artery detection with Doppler ultrasound.

Materials and methods: Ultrasonographic measurements were performed with 11 MHz linear transducer probe for 40 subjects comprising of 20 tobacco chewers and 20 non chewers.

Results: Study group showed increased thickness both on right and left side masseter muscle and also in relaxed and contracted state when compared to control group. The Contracted masseter muscle thickness was more than relaxed state in both groups which was highly significant. No statistically significant differences between right and left side muscle lengths and artery detection were obtained.

Conclusion: Our study emphasizes the detection of masseter muscle hypertrophy in tobacco chewers which would otherwise lead to muscle pathology. However more studies have to be carried out owing to high prevalence of tobacco chewing in India. Also, evaluating more blood flow parameters other than artery detection alone in the muscle is recommended to determine vascularity.

Keywords: Tobacco chewing, Masseter muscle hypertrophy, Ultrasonography, Doppler ultrasound

Tobacco was introduced by the Europeans into South Asia in the 1600s, for pipe smoking and also as snuff. An estimate of the number of betel quid users globally is 600 million. Current male chewers of betel quid with tobacco in case-control studies in India had relative risks of oral cancer varying between 1.8-5.8.¹

Tobacco in India, is used in a variety of forms such as smoking, chewing, local applications, drinking and gargling, leading to detrimental health effects such as increased incidence of and mortality from cardiovascular diseases, cerebrovascular diseases, respiratory diseases and cancer, in addition to detrimental reproductive outcomes, dental and oral diseases.

Betel-quid chewing which is a mixture of areca nut, slaked lime, catechu, other spices and condiments wrapped in a betel leaf is a popular, socially accepted, ancient custom in India and the introduction of tobacco reinforced this practice. The introduction of commercial pan masala, dehydrated and non-perishable powdered areca nut slaked lime, catechu, cardamom and other flavouring and perfuming agents with or without tobacco available in attractive sachets or tins has enhanced the sale and use of smokeless tobacco.

The carcinogenic effect of betel-quid and pan masala has lead to one of the highest incidence and mortality rates of oral cancer with 83, 000 incidence cases and 46,000 deaths annually in India². Chronic hypertrophy of the muscles of mastication can cause increased salivary flow in chewers.³

The jaw muscles control the position and motion of the mandible and create forces at the teeth and temporomandibular joints. The masseter muscle, one of four muscles of mastication, participates in a wide variety of activities including mastication, swallowing and speech. This diversity of function requires coordination of motor output elements of masticatory muscles (i.e., compartments) along with appropriate activation of tongue, facial and oropharyngeal muscles. The complex internal tendon architecture subdivides the masseter into multiple partitions that can be further subdivided into neuromuscular compartments.⁴

Jaw muscles are versatile entities that are able to adapt their anatomical characteristics, such as size, cross-sectional area, and fibre properties to altered the functional demands. The resistance training of a skeletal muscle, for example by means of repeated isometric contraction and relaxation, causes an increase in the thickness of the muscle and enhances muscular strength.⁵

The field of medical imaging, stimulated by advances in digital and communication technologies, has grown tremendously. Among the imaging techniques ultrasonographic imaging is a method that has been proven to be capable of providing information by depicting muscle structural alterations. Ultrasound provides uncomplicated and reproducible access to parameters of jaw muscle function and the interaction within the cranio-mandibular system and this method represents a considerable improvement over conventional

methods for assessing masseter thickness, particularly in terms of clinical availability and cost.

Ultrasound has been described as an accurate and reliable imaging technique for measuring the thickness and cross sectional area of the masticatory muscles.⁶

In spite of high prevalence of tobacco chewing in India not many studies have been done to assess muscle changes in Tobacco chewers. In our study we assess the thickness of Masseter muscle and detection of Branch of transverse facial artery in tobacco chewers and compare them with those in healthy controls.

AIM:

To assess changes in Masseter muscle using Ultrasonograph equipped with 11 MHz linear array transducer in patients with the habit of tobacco chewing for more than 2 years with a frequency of more than 2 packets per day against subjects with no tobacco chewing habit.

OBJECTIVES:

- To measure Masseter muscle length as an index of muscles thickness in Tobacco chewers using Ultrasonography.
- To measure Masseter muscle length as an index of muscles thickness in non chewers using Ultrasonography.
- To compare Masseter muscle length in Tobacco chewers and non chewers using Ultrasonography.
- To compare the muscle length between Right and Left sides in Control and study groups.
- To assess the detection rate of branch of transverse facial artery in tobacco chewers using colour Doppler ultrasonography.
- To assess the detection rate of branch of transverse facial artery in non chewers using colour Doppler ultrasonography.
- To compare detection rate in tobacco chewers and non chewers using colour Doppler ultrasonography.

HISTORICAL OVERVIEW OF TOBACCO IN INDIA⁷

The History of global tobacco trade is integrally linked with the history of India. It was to discover a sea route to this fabled land, reputed for its spices, silk and gems, that Christopher Columbus set sail in 1492. His wayward journey took him instead to America. This discovery of the 'New World' was accompanied by the discovery of tobacco by Portuguese sailors. This plant, treasured by the American 'Indians' for its presumed medicinal and obvious stimulant properties, was eagerly embraced by the Portuguese who then moved it to the Old World of Europe. Even though their quest for easy access to Indian spices was delayed by some years, the Europeans did not fail to recognize the commercial value of this new botanical acquisition.

When the Portuguese eventually did land on India's shores, they brought in tobacco. They introduced it initially in the royal courts where it soon found favour. It became a valuable commodity of barter trade, being used by the Portuguese for purchasing Indian textiles. The taste for tobacco, first acquired by the Indian Royals, soon spread to the commoners and, in the seventeenth century, tobacco began to take firm roots in India. Thus, tobacco travelled to the real Indians from their curiously named American cousins, through the medium of European mariners and merchants who sailed the seas and spanned the continents in search of new markets and colonies. It was with the establishment of British colonial rule, however, that the commercial

dimensions of India's tobacco production and consumption grew to be greatly magnified.

While tobacco chewing was practised for many centuries, commercial production and marketing have been markedly upscaled recently, with the introduction of Gutkha. The rate of growth of consumption of gutka has overtaken that of smoking forms of tobacco.

The economics of tobacco, which introduced it into India and entrenched it during the colonial rule, also provided a compelling reason for continued state patronage to the tobacco trade, even in free India. The ready revenues that bolster the annual budgets, the ability to export to a tobacco-hungry world market and the employment opportunities offered to millions provided the rationale for encouraging tobacco, both as a crop and as an industry

REVIEW OF SMOKELESS TOBACCO USE IN INDIA⁸

Tobacco is used in a number of smokeless forms in India, which include betel quid chewing, mishri, khaini, gutka, snuff, and as an ingredient of pan masala.

Generally sun or aircured smokeless tobacco can be used by itself in unprocessed, processed or manufactured form. It can be used with lime, with areca nut or in a betel quid (pan). The use of unprocessed tobacco, the cheapest form, varies in different parts of India. It is sold as bundles of long

strands in Kerala or as leaf tobacco (hogesoppu) in Karnataka. Kaddipudi are cheap ‘powdered sticks’ of raw tobacco stalks and petioles, used in Karnataka. Sometimes this powder is formed into bricks or blocks mixed with jaggery (solid molasses) and water. Gundi, also called kadapan, is a mixture of coarsely powdered tobacco with coriander seeds, other spices and aromatic, resinous oils, popular in Gujarat, Orissa and West Bengal. Kiwam or qiawam, used mainly in north India and Pakistan, is a thick paste of boiled tobacco mixed with powdered spices such as saffron, cardamom, aniseed and musk, and is also available as granules or pellets. A commercial mixture of tobacco, lime and spices is zarda. It is typically flavoured with cardamom and saffron and often chewed in betel quid, and is popular in north India, Pakistan and Bangladesh. Pattiwala is sun-dried, flaked tobacco with or without lime, used mainly in Maharashtra and several north Indian states.¹

Betel quid is a combination of betel leaf, areca nut, slaked lime, tobacco, catechu and condiments according to individual preferences.

Khaini consists of roasted tobacco flakes mixed with slaked lime. This mixture is prepared by the user keeping the ingredients on the left palm and rubbing it with the right thumb. The prepared pinch is kept in the lower labial or buccal sulcus. Its use is common in eastern India.

Mawa is a mixture of areca nut, tobacco and slaked lime and is chewed. Its use is common in rural areas of Gujarat province. It is quite popular among the young population of ages 15-19 years.

Snuff is a black-brown powder obtained from tobacco through roasting and pulverization. Snuff is used via nasal insufflation and is popular in eastern parts of the country. It is also applied on the gum by finger (this practice is usually initiated as a dentifrice) in the Western India, where it is known as *bajar* and *mishri*.

Gutka is a manufactured smokeless tobacco product (MSTP), a mixture of areca nut, tobacco and some condiments, marketed in different flavours in colourful pouches.

Pan masala is a betel quid mixture, which contains areca nut and some condiments, but may or may not contain tobacco. The mixture is chewed and sucked.

Unlike cigarettes, tax levied on pan masala is low. Low cost and not being associated with smoke have led to an enormous increase in the use of all types of areca nut and smokeless tobacco among the Indian population including adolescents. It has also been promoted as a “post meal mouth freshener”, making it quite popular.

Initially, it was more popular in the Northern India, but with a massive advertising, it is now being used all over the country.

Pan masala is as harmful as smoking, although the nature of harmful effects are different. Its use has been associated with high risk of oral cancer and sub mucous fibrosis in mouth, which also has a high potential for cancer

development. It is made by the use of waste tobacco, mid-ribs of tobacco leaves and floor sweepings from cigarette factories. It is available in the forms of small packets and cans, sold at affordable prices with attractive, shiny colored wrappings.

Pan masala was initially popular in the urban segment only, but over the last few years, it has been consumed in rural areas as well. According to the most recent Government of India's National Sample Survey data, there are 184 million tobacco consumers in India. About 40% of them use smokeless tobacco.

EPIDEMIOLOGY OF TOBACCO CHEWING IN INDIA⁹

Adult prevalence of smokeless tobacco use varies greatly among the countries in South East Asian region but is substantial in six countries of the region. For men it varies from 1.6% in Thailand, 32.9% in India, and 51.4% in Myanmar. The high prevalence is because all these countries lie in Betel quid belt, the area where betel quid has been used for many centuries.

The prevalence of betel quid chewing has been studied in parts of Asian countries in adults aged > 15 years among the three SEAR countries surveyed the prevalence of betel quid chewing was found to be highest in Nepal (Men 43.6%, Women 34.9%)

According to the results of Global adults tobacco survey in India Tobacco with lime (Khaini) is the most common form of tobacco chewing.

According to WHO NCD STEPS¹⁰ survey conducted between 2003-2004 in city of Chennai in age group of 15-65 years, the most common form of tobacco chewing was found to be Gutkha and percentage of daily smokeless tobacco use among males and females was found to be 20% and 8.1% respectively.

An estimate of the number of betel quid users globally is 600 million. Smokeless tobacco users in India and Pakistan together have been estimated to number 100 million¹¹. By 2020 tobacco consumption has been projected to account for 13% of all deaths in India.¹²

Rani, Bonu, Jha, et al (2003)¹³ estimated the prevalence and the socioeconomic and demographic data correlates of tobacco consumption in India. Design of study was Cross sectional, nationally representative population based household survey. 315,598 individuals 15 years or older from 91,196 households were sampled in National Family Health Survey-2 (1998–99). Data on tobacco consumption were elicited from household informants. Prevalence of current smoking and current chewing of tobacco were used as were used as outcome measures. Thirty per cent of the population who are 15 years or older among which 47% men and 14% of women chewed tobacco, which translates to almost total of 195 million people out of which 154 million are men and 41 million are women in India.

Rooban et al (2010)¹⁴ estimated the prevalence, Socioeconomic, demographic data on chewable smokeless tobacco users in India. Among the 74,369 males aged 15-54 years who were sampled in the National health survey 3 [2005, 2006] thirty four percent of study population who were 15 yrs. or older used chewable smokeless tobacco.

Sreeramareddy et al. (2011)¹⁵ A secondary data analysis of 2006 Nepal Demographic and Health Survey (DHS) was done. A representative sample of 9,036 households was selected by two-stage stratified, probability proportional to size (PPS) technique. They constructed three outcome variables 'tobacco smoke', 'tobacco chewer' and 'any tobacco use' based on four questions about tobacco use that were asked in DHS questionnaires. Socio-economic, demographic and spatial predictor variables were used. Total number of households, eligible women and men interviewed was 8707, 10,793 and 4397 respectively. The overall prevalence for 'tobacco chewing' were 14.6% (95% CI 13.5, 15.7) respectively. Prevalence among men was significantly higher for 'tobacco chewing' (38.0% versus 5.0%). By multivariate analysis, older adults, men, lesser educated and those with lower wealth quintiles were more likely to be using all forms of tobacco.

Chemical composition of Betel nut

Although a considerable number of chemical constituents are present in betel quid only pyridine alkaloids and polyphenols have gained considerable attention as these substances have clinical implications.

Arecoline forms the major alkaloid and Arecaidine, Guvacaine and Guvacoline and Arecolidine constitute minor alkaloids.

The addictive nature of areca nut has been suggested to be due to inhibition of GABA uptake in Central nervous system by areca alkaloids.

MN Awang¹⁶ estimated arecoline contents in commercial areca nuts available in Bombay and comparing with arecoline contents in Kerala and Mysore reported higher concentrations of Arecoline in the nine samples studied and concluded that variations may be due to variations in raw materials and processing methods.

A REVIEW OF MASSETER MUSCLE AND ELECTROMYOGRAPHY IS PRESENTED

The Masseter muscle is essential for mastication and play an important part in craniofacial growth. They contribute to dental and articular forces, deform the mandible, and, like other tissues, are subject to disorders, often manifested as pain. Their contraction is controlled by the nervous system, and their general structure and function contribute to craniofacial biology, but there has been little appraisal of their internal organization. Most of these muscles are not simple; they are multipennate, complexly layered, and divided by aponeuroses. This arrangement provides substantial means for differential contraction. In many ways, jaw muscle fibres are intrinsically dissimilar from

those found in other skeletal muscles, because they are arranged in homogeneous clusters and generally reveal different histochemical profiles.¹⁷

Muscle fibres usually attach directly to bone or to cylindrical, ovoid, or elongate tendons. However, complex skeletal muscles often contain quite large internal aponeuroses (sheets of compacted collagen fibres) to which muscle fibres attach, and it is common for these aponeuroses to differ in orientation and size within the same muscle. Thus, fibres may lie either parallel to the line of action of the muscle or at an angle to it, attached obliquely to aponeuroses. When groups of fibres are angled, that is, when they fan out on either side of a central tendon or aponeurosis, they look like a feather ("penna" L.); hence, the term "pennate" (pinnate).¹⁷

Fibres in parallel fibered muscles produce translational motion exclusively. Those in pennate muscles rotate about their origins, increasing the angle of pennation as they shorten. The attached tendon or tendon sheet then translates in the desired direction. If one of the attachments is to a tendon sheet and the other to an area of bone, both translation and rotation of bone and/or aponeurosis are possible due to an induced couple. Patterns of pennation vary between muscles, which are thus classified as unipennate, bipennate, and multipennate. Examples of all three can be found in the jaw muscles. Pennation is advantageous in muscles required to produce power under spatial constraints and when some loss of muscle shortening can be tolerated.

The increased cross-sectional area achieved by packing short fibres at acute angles to an internal septum offers a major increase in muscle tension.

Though information regarding fetal development of the masseter is sparse. Unpublished observations suggest that most major structural elements are in place by 18 weeks as stated by Tonndorf, personal communication. The muscle comprises a superficial part, which arises via a thick, multileaved aponeurosis from the anterior two thirds of the lower border of the zygomatic arch as far anteriorly as the zygomatic process, and that inserts from the angle of the mandible anteriorly to the ascending ramus; an intermediate part that arises from the central, medial third of the zygomatic arch and lower border of its posterior third; and a deep part arising from the deep surface of the arch. Both the intermediate and deep parts insert, respectively, on the central and upper parts of the ascending ramus to the level of the coronoid process. The masseteric nerve separates the deep and intermediate parts, while the masseteric artery separates the intermediate and superficial layers.¹⁷

Viewed coronally and from behind, the masseter reveals its multipennate character, with oblique subsets of fibres. Most of these insert into the interleaved aponeuroses. Overall, the muscle's fibres differ in length, averaging around 26 mm, and ranging from 14 to 19 mm at their shortest, to 30 to 38 mm at their longest¹⁸. Some fibres, particularly those radiating from the ends of the aponeurotic sheets, insert directly into ramal or zygomatic arch bone. Observed parasagittally, these same fibre collections would appear to be

aligned roughly parallel and obliquely from zygomatic arch to the mandible, but they are also angled mediolaterally through the plane of view, and do not extend the full distance, as each attaches to an aponeurosis. This arrangement makes it very difficult to establish actual pennation angles from region to region. Although they are considered to be about 20° or less, distinction should be made between angles of origin and insertion relative to the zygomatic arch and ramus and angles expressed relative to the various aponeuroses within the muscle.¹⁸

The longest fibres are found anteriorly, where they are about 35% longer than those found posteriorly when the teeth are in contact. The deeper fibres are about 5% shorter than the superficial. Tendon length does not alter much within the superficial layers, averaging about 30 mm. Tendons in the deep muscle layer, however, are about 35% shorter than those superficially.

The architecture of the central and deep parts of the muscle remains the most ambiguous. In addition to the two or three septa descending from the zygomatic arch, and up to two ascending from the ramus, there is at least one tendon that terminates a fan-shaped collection of fibres in the deep, upper part. This region is reminiscent of a small temporalis muscle. These differences in regional morphology clearly imply functional differentiation. It is logical to presume that there are practical advantages to be gained by an internal architecture as complex as this. For example, the posterior laminations attached at different heights on the ramus could permit muscle layers to slide

over one another during jaw rotations that involve different trajectories of movement by various parts of the ascending ramus, and that must take place in three dimensions of space.¹⁷

Masseter activity is known to be highest when chewing is carried out on the ipsilateral side, particularly near the intercuspal position. From this we might conclude that during chewing the masseter also exerts strong laterally and upwardly directed forces on the ipsilateral ramus, while the latter moves roughly perpendicular to this direction, that is, the muscle acts as an efficient generator of dental force on the ipsilateral side, but in this case does not contribute to medial movement of the ramus (which is presumably effected by other muscles). This notion does not preclude the selective activation of fibres on each side of intramuscular aponeuroses, modifying the direction of pull as needed and introducing strong intramuscular force couples.

The further the mandible is from the intercuspal position, the more sharply defined are regional differences in the three-dimensional "starting" locations of muscle insertion sites, and the more likely the need for differential muscle activity.¹⁷

Ju-Young Lee¹ et al (2012)¹⁹

The authors carried out a study to elucidate the topographic anatomy of the masseter muscle, focusing on its tendinous digitation. Sixty-five adult faces (113 sides) were dissected. Five parameters, including the lengths,

widths, and thickness of the muscle, were measured. The number and morphology of tendinous digitations were also investigated. The length and width of the masseter muscle were longer and wider in male specimens than in female specimens. The number of masseter muscle tendinous digitations was predominantly two in males and three in females. The length of the tendinous digitations tended to be about three-quarters of that of the muscle. The second tendinous digitation was the longest in male specimens, while the first tendinous digitation was the longest in females.

Definition of EMG²⁰

"Electromyography (EMG) is an experimental technique concerned with the development, recording and analysis of myoelectric signals. Myoelectric signals are formed by physiological variations in the state of muscle fibre membranes."

Benefits of EMG

- EMG allows to directly “look” into the muscle
- It allows measurement of muscular performance
- Helps in decision making both before/after surgery
- Documents treatment and training regimes
- Helps patients to “find” and train their muscles

- Allows analysis to improve sports activities
- Detects muscle response in ergonomic studies

The Motor Unit

The smallest functional unit to describe the neural control of the muscular contraction process is called a Motor Unit. It is defined as “...the cell body and dendrites of a motor neuron”. The multiple branches of its axon and the muscle fibres that innervates it. The term units outlines the behaviour, that all muscle fibres of a given motor unit act “as one” within the innervation process.²⁰

Van Eijden et al (1993)²¹ studied Electromyographic (EMG) activity in the human masseter muscle which was registered from six different sites, in the anterior, middle, and posterior regions of the superficial and deep layers of the muscle, during static clenching tasks (intercuspal and incisal), selected jaw movements (alternating protrusion/retrusion, right/left latero-deviation, and open/close excursions), and unilateral chewing on right and left sides. Peak-EMG amplitudes and the timing of the peaks were compared. Activity in the regions of the deep masseter was either higher (in mastication and intercuspal open/close excursions) or lower (incisal clenching) than the activities in the superficial masseter. Superficial and deep masseter also differed in their timing of peak EMG: During chewing, peak activity passed from superficial to deep in the balancing-side muscle, and from deep to superficial on the

chewing side. During free latero-deviations, peak activity started in the deep masseter, when the jaw moved to the right side (i.e., the side of the muscle), and then passed to the superficial regions, after the jaw movement was reversed to the left side. In addition, within the deep masseter there existed clear anteroposterior differences in activation level (during incisal clenching and open/close excursions) and in timing (during latero-deviation). Such a differentiation of activity was not found in the superficial masseter.

REVIEW OF MUSCLE HYPERTROPHY²²

The skeletal muscle is characterized by several peculiarities that make it one of the more astounding tissues of the body. One of its unusual characteristics resides in its being highly heterogeneous in fibre type, so that it may be looked at as a patchwork of rather different cells; another important feature is its being made up of multinucleated cells, as a result of cell fusion events occurring during development. These factors contribute to make the skeletal muscle the second more plastic tissue of the body. In fact, skeletal muscle structure and function can adapt with surprisingly easiness to environmental changes and to different stimuli, ranging from stimuli modifying its contractile activity (inactivity, endurance exercise, denervation, electrical stimulation), stimuli modifying imposed load (resistance exercise, unloading, microgravity) and to other environmental factors such as heat, hypoxia, nutrient availability, growth factors and inflammation mediators

Signalling in Muscle Hypertrophy²³ Muscle performance is influenced by turnover of contractile proteins. Production of new myofibrils and degradation of existing proteins is a delicate balance, which, depending on the condition, can promote muscle growth or loss. Protein synthesis and protein degradation are co-ordinately regulated by pathways that are influenced by mechanical stress, physical activity, availability of nutrients, and growth factors. Cell size is determined by a balance between new protein accumulation and degradation of existing proteins. Genetic studies in both drosophila and mammals have shown that pathways controlling protein synthesis and protein breakdown have an important role to determine cell size. The two processes are tightly regulated and interrelated. The first level of connection occurs during protein synthesis when the quality control of the cell degrades proteins that are not correctly folded. At a further level, protein degradation systems determine the half-life of protein and, in muscle, are required to replace sarcomeric proteins as a consequence of changes in muscle activity. Both systems need ATP, and muscle energy level is one of the cellular check points that decide either to promote growth and hypertrophy or activate protein breakdown and atrophy. Importantly, the proteolytic systems can produce alternative energy substrates that are used by the cell to maintain internal homeostasis in conditions of energy stress. Recent findings provide a new view, which considers the growth-promoting pathways and the proteolytic systems co-ordinately regulated.

The growth of skeletal muscle mass, like the mass of any other tissue, depends on protein turnover and cell turnover. Cellular turnover plays a major role during muscle development in embryo. Moreover satellite cell incorporation into the growing fibres takes place during postnatal muscle growth concomitantly with increased protein synthesis. The activation of satellite cells is important for maintaining a constant size of each nuclear domain (quantity of cytoplasm/number of nuclei within that cytoplasm). Unlike young muscle, the contribution of cellular turnover to homeostasis of adult fibres is minor, and its role in hypertrophy has even been recently debated. In adult muscle, the physiological conditions promoting muscle growth, therefore, do so mainly by increasing protein synthesis and decreasing protein degradation. However satellite cells are activated in compensatory hypertrophy, and addition of new nuclei to the growing fibre seems to be required for extreme hypertrophy. The pathways controlling cellular and protein turnover are different, and their contribution to muscle hypertrophy has to be considered during the interpretation of data resulting from studies with transgenic animals. Loss and gain of function studies in which the transgene is perturbed early during postnatal growth might affect cellular turnover significantly more than protein synthesis. Results could be completely different if the same pathway is acutely perturbed in adult muscle age when the role of protein turnover is dominant.²³

Insulin derived Growth factor 1 [IGF1-AKT signalling and the control of muscle growth]

IGF1 is among the best characterized muscle growth-promoting factors. In addition to circulating IGF1, mainly synthesized by the liver under GH control, local production by skeletal muscle of distinct IGF1 splicing products has recently raised considerable interest. A specific IGF1 splicing product is important for load- and stretch-induced adaptations in skeletal muscle. Increased IGF1 gene expression has been demonstrated following functional overload induced by elimination of synergistic muscles. Muscle-specific overexpression in transgenic mice of an IGF1 isoform locally expressed in skeletal muscle results in muscle hypertrophy and, importantly, the growth of muscle mass matches with a physiological increase of muscle strength. Moreover even acute ectopic expression of IGF1 in adult muscles by electroporation is sufficient to promote muscle hypertrophy. Although these results suggest an autocrine/paracrine role for local IGF1 in activity-dependent muscle plasticity.²³

Akt thymoma

Akt activation is induced by IGF1 and insulin through the generation of phosphatidylinositol-3,4,5 triphosphates produced by PI3K, which is opposed by the activity of the phosphatase PTEN and SHIP2. Phosphatidylinositol-3,4,5 triphosphates recruit Akt to the plasma membrane by binding to its NH2-terminal pleckstrin homology domain. At the

membrane, Akt is phosphorylated on separate residues by at least two distinct kinases, PDK1 and the mTOR-Rictor complex. In mammals, there are three Akt1 (PKB α), Akt2 (PKB β) and Akt3 (PKB γ) which appear to have distinct functions. In skeletal muscle, Akt1 and Akt2 are expressed at higher levels compared with Akt3, which is mainly expressed in the brain. Exercise in vivo is associated with activation of Akt1 but not Akt2 and Akt3 kinases in contracting muscles. Akt activity is also increased in response to hormonal and growth factor stimulation, in particular insulin is known to activate Akt2, whereas IGF1 activates primarily.

Akt1 taken together with other observations, suggest that it is a major mediator of skeletal muscle hypertrophy.

mTOR-S6K and the control of protein synthesis

Two major downstream branches of the Akt pathway, which are relevant to muscle hypertrophy, are the TOR pathway, which is activated by Akt, and glycogen synthase kinase 3 β (GSK3 β) which is blocked by AKT; both of them control protein synthesis. The kinase mTOR (mammalian target of rapamycin) has recently emerged as a key regulator of cell growth that integrates signals from growth factors, nutrients, and energy status to control protein synthesis and other cell functions.²³

Myostatin and the cellular turnover

Myostatin, a member of the TGF-family, is expressed and secreted predominantly by skeletal muscle and functions as a negative regulator of muscle growth. Mutations of the myostatin gene lead to a hypertrophic phenotype in mice, sheep, and cattle, and a loss of function mutation in the human myostatin gene was also found to induce increased muscle mass . The increase in muscle mass is a consequence of hyperplasia, which is an increase in cell number, and hypertrophy, which is an increase in cell size. The hyperplasia suggests an activation of muscle stem cells and in fact, the myostatin pathway influences Pax 7, MyoD, and myogenin expression inhibiting satellite cell activation and differentiation.²³

Beta adrenergic and mechanical sensors

Among the hormonal responses increased by exercise, the acute elevations in catecholamines are especially interesting with respect to changes in muscle phenotype. Beta-agonists such as clenbuterol, acting through 2-adrenoreceptors, are known to cause muscle hypertrophy and a slow-to-fast fibre-type switch. Interestingly, some effects of catecholamines could be mediated by local production of IGF-I and IGF-II by skeletal muscle. An attractive emerging concept in muscle biology is that signals dependent on muscle activity, and specifically on mechanical load, may arise in the sarcomere the basic unit of the contractile machinery of striated muscles, and from there transmitted to the nucleus to affect gene expression.²³

INFLUENCE OF INFLAMMATORY CELLS ON MUSCLE HYPERTROPHY²⁴

Most research on muscle hypertrophy has concentrated on the responses of muscle cells to mechanical loading; however, a number of studies also suggest that inflammatory cells may influence muscle hypertrophy. Neutrophils and macrophages perform many functions that may be important, including phagocytosis, production of free radicals, cytokines and growth factors. Neutrophils and macrophages accumulate in skeletal muscle following increased mechanical loading, and we have demonstrated that macrophages are essential for muscle hypertrophy. Mechanical loading of skeletal muscle can initiate an inflammatory response, characterized by the accumulation of neutrophils and macrophages in skeletal muscle and the expression of various cytokines. Classically, the function of neutrophils and macrophages has been restricted to the removal of damaged tissue via phagocytosis. However, emerging evidence on their contribution to various physiological responses of skeletal muscle cells both in vitro and in vivo, indicate that neutrophils and macrophages play a far more complex role in skeletal muscle than simply removing damaged tissue. Physiological responses associated with inflammation include dilatation and increased permeability of blood vessels, increased blood flow, exudation of fluid, and leukocyte migration to the area of injury or infection emerging evidence indicates that in skeletal muscle, cellular events associated with the inflammatory response, namely those

associated with nonspecific (innate) immunity, can occur in the absence of overt injury.

NEUTROPHILS AND MACROPHAGES: THEIR POSSIBLE FUNCTIONS IN MUSCLE HYPERTROPHY²⁵

Neutrophils and monocytes/macrophages are inflammatory cells that develop in the bone marrow and are released into the circulation to serve as sentinels of the innate immune system. A variety of molecules can be released from cells residing in skeletal muscle (e.g., skeletal muscle cells, endothelial cells, and macrophages) that can call inflammatory cells into action by promoting their migration to and within skeletal muscle after mechanical loading. For example, chemokines (e.g. IL-8, GRO α , β) are potent neutrophil chemoattractants, and chemokines (e.g. MCP-1, MIP-1 α , β) are potent monocyte/macrophage chemoattractants. Upon their arrival in skeletal muscle, neutrophils and macrophages could influence muscle hypertrophy via their capacity to perform phagocytosis, and to produce free radicals, cytokines and growth factors.

Possible roles of phagocytosis in muscle hypertrophy include removal of damaged extracellular matrix as well as the removal of damaged, necrotic and/or apoptotic cells from skeletal muscle. The production of free radicals by inflammatory cells in skeletal muscle could have multiple functions. In the context of phagocytosis, free radicals released into the phagolysosome aids in the degradation of endocytosed material. In addition, the release of free

radicals from inflammatory cells into the extracellular fluid may also cause “collateral damage” to adjacent healthy tissue. Indeed, free radicals produced by inflammatory cells are known to damage different cell types, including skeletal muscle cells. Downstream products of hydrogen peroxide (e.g., hypochlorous acid and hydroxyl radical) appear to be most injurious to differentiated skeletal muscle cells. However, other non-inflammatory cell types that are found in skeletal muscle (e.g., endothelial cells, fibroblasts, and skeletal muscle cells) can also produce cytokines and little is known about the cellular sources of cytokines during mechanical loading.

Many factors produced by inflammatory cells are also known to have biological functions that are not directly related to the inflammatory response per se. For example, a number of growth factors (e.g. IGF-1, FGF, HGF, VEGF, TGF β) influence the proliferation, migration and metabolism of different cells, including those that contribute to muscle hypertrophy. Indeed, soluble factors produced by monocytes/macrophages are known to induce proliferation and differentiation of skeletal muscle cells.^{24,25}

MASSETER MUSCLE HYPERTROPHY

The Condition was first described by Legg in 1880.²⁶ Masseter muscle hypertrophy is an asymptomatic, benign enlargement of one or both masseter muscles. It is a relatively rare condition, with around 130 cases reported in the literature since the first described. It is most commonly seen in late adolescence and early adulthood. Studies show that the mean age of

occurrence was 30 years. There are several theoretical considerations about the etiology of masseter muscle hypertrophy, but it still remains unclear. Several authors claim that emotional stress results in chronic forceful clenching of the jaws and bruxism, which cause a work hypertrophy of the muscle.²⁷

The bone spurs at the mandible angle are commonly associated findings and they can be observed in the anteroposterior radiograph. However, Bloem and Hoof stated that approximately 20% of normal people have this finding and that it cannot be considered a diagnostic aid ²⁸. It was reported that bone spurs are caused by periosteal irritation and new bone deposition responding to increased forces exerted by the muscles bundles.

The differential diagnosis included parotiditis, parotid tumor, lipoma, benign or malignant muscle tumors, vascular tumors, benign and malignant mandible tumors. The correct diagnosis is more difficult in unilateral cases and requires a differential diagnosis with parotid gland alterations, which justifies the need of performing a sialography in order to discard this possibility. Therapy for masseteric enlargement is usually unnecessary. Non-surgical modalities of treatment include reassurance, tranquilizers or muscle relaxants, psychiatric care and injection of very small doses of botulinum toxin type A.²⁹

ULTRASONOGRAPHY

History³⁰

In the sixth century B.C. the Greek philosopher Herakleitos of Ephese (570-480) stated that war laid the foundation of everything. Recent examples have demonstrated this statement in the field of medical imaging: they were supplied throughout the history of the discovery of ultrasound.

From bat to medical ultrasound

In the animal world, whales, dolphins, and bats have been moving around for thousands of years using ultrasound. It is not until 1794 that man discovered the existence of this phenomenon. The Italian naturalist Lazzaro Spallanzani (1729 - 1799) carefully studied bats and discovered that they didn't use their visual capacity to move around but rather their acoustic capacity. This capability enables them to avoid obstacles in absolute darkness. It took another century before man could generate ultrasound. In 1880, the brothers Pierre (1859- 1906) and Jacques Curie (1855- 1941), who analyzed the piezoelectric qualities of crystals, discovered how to produce ultrasound. The first practical application is ascribed to Sir F. Galton (1822-1911) who used an ultrasonic whistle to call his dog.

The sinking of the Titanic in 1912 and the great loss of ships torpedoed by German submarines in World War 1, led to the perfection of specific targeting and navigational technology. P. Langevin (1872-1946), P. Curie's

student, recalled his mentor's discovery and used the technology to locate a submarine that was sank (April 23-1916) in shallow water.

After the war, the research was put aside and a little forgotten. It was again resumed at the event of World War II. These circumstances gave rise to the Sonar (Sound Navigation and Ranging) that was frequently used during the second World War. However, the use of low energy ultrasound already existed before the war in industrial applications.

An attempt to medical application was exercised in 1942 by the Austrian neuropsychiatrist K. Dussik (1908), who was assisted by his brother, the physicist. Using a continuous ultrasonic emitter, they attempted to interpret the bizarre images from the patient's brain. The resulting images led to an enormously controversial interpretation. This method was abandoned when it became evident that the research through bony structures (the skull) was a contraindication for ultrasonic examinations.

It is generally assumed that G. Ludwig, internist and former US navy military physician, and his assistant F. Struthers, a US Navy engineer, were the first who could precisely detected bile stones they had put in beefsteaks. However, the most prominent figure appears to be the American radiologist D. Howry (1920-1969). His team created the first live ultrasonic image using declassified military material taken from the gun turret of a B29 bomber. The patient was seated on an old dentist chair and was submerged in the water-containing gun turret from the neck down. The necessity of aquatic

submergence seriously diminished the application possibilities, for example with patients who had undergone surgery. The search for procedures, which are too numerous to name, continued to avoid the submergence in water and to produce more centered and focused ultrasonic waves.

A decisive step was taken in 1958 with the introduction of contact ultrasound by the Scottish gynecologist. Donald (1910-1987). Instead of aquatic submergence, he used a viscous gel, a substance still in use today. This procedure was immediately applied to the medical world, especially gynecology and obstetrics.

The old dream of visualizing medical ultrasounds and their echoes was finally realized.

PHYSICS OF ULTRASOUND³¹

Ultrasound consists of mechanical waves with frequencies above the upper auditory limit of 20 kHz. Frequency is equal to the number of wave cycles produced each second, and medical US devices commonly use longitudinal waves with a frequency range of about 2–15 MHz. Mechanical waves must travel through some physical medium like air, water, or tissue. These waves correspond to regions in the medium where pressure is alternately higher than and lower than the resting or ambient pressure. Where pressure is high, the medium is squeezed or compressed; where pressure is low, the medium is stretched or rarefied. The medium moves in an oscillatory

manner, alternating between states of compression and rarefaction. Each small element of the medium moves back and forth about its resting location but does not undergo any net motion as the wave propagates. The term longitudinal refers to waves that cause oscillatory motion of the medium in the same direction as the direction of wave propagation. Transverse waves (shear waves), in which the medium oscillates in a direction perpendicular to the propagation direction, are rapidly attenuated in tissue and so do not play a direct role in medical B-mode imaging.

Another commonly encountered acoustic variable is the acoustic intensity, which is defined as the power per unit cross-sectional area of the ultrasound pulse. Ultrasound that is tightly concentrated or focused has a higher intensity than ultrasound emitted with the same power but spread over a broader area. Intensity is correlated with the likelihood of bio effects resulting from exposure to ultrasound. Echo pressure amplitudes can vary by a factor of 10^5 or greater, so relative pressure and intensity levels are more conveniently discussed in terms of decibels. Relative pressure amplitude expressed in decibels equals $20 \cdot \log (P_2/P_1)$, where P_1 and P_2 are the two pressure amplitudes being compared. These might correspond to the pressures of an initial ultrasound pulse (P_1) and an echo from some anatomic structure (P_2). Similarly, relative intensity expressed in decibels equals $10 \cdot \log (I_2/I_1)$.

The difference in the leading constants in the two expressions (“20” and “10”) is due to the fact that intensity is proportional to the square of the pressure amplitude.

Ultrasound pulses travel through biologic tissues with an average velocity (c) of about 1,540 m/sec. The actual velocities in specific tissues vary about this average. For example, the sound speeds of fat, amniotic fluid, kidney, muscle, and skull bone are about 1,450, 1,540, 1,565, 1,600, and 4,080 m/sec, respectively. US scanners commonly assume that ultrasound travels through all tissues with a speed of 1,540 m/sec.

This assumption is used to compute the depth (D) at which detected echoes were produced by rearranging the definition of velocity: $D = (\text{time from pulse generation to echo detection}) \cdot c/2$. This expression is commonly referred to as the range equation. The factor of “2” results from the fact that the total round-trip path length includes the travel of the pulse from the transducer to the reflector, and then the travel of the echo back from the reflector to the transducer.

Interactions of Ultrasound with Tissue³¹

Echo generation results from the interaction of the incident ultrasound pulse with structures in the tissue medium, and there are several specific types of interaction that contribute to this process. Important to all of them is a tissue property called the acoustic impedance (Z). A simplified definition is $Z = \rho c$,

where ρ is the density of the tissue. This quantity is more properly called the specific acoustic impedance of the medium. This quantity is more properly called the specific acoustic impedance of the medium. A partially reflected echo that travels back toward the transducer and a partially transmitted pulse that travels deeper into the patient. This type of reflection is called specular reflection. The intensities of the reflected and transmitted pulses sum to the intensity of the original incident pulse (i.e, energy is conserved in this interaction). For normal or 90° incidence, the intensity of the reflected pulse (I_r) as a fraction of the incident intensity is given by the expression $I_r = (Z_2 - Z_1)^2 / (Z_2 + Z_1)^2$. This quantity is called the intensity reflection coefficient. The intensity of the reflected echo increases with increasing impedance difference between the two tissues. If the tissues have identical impedance, no echo results. Interfaces between tissues (excluding lung and bone) generally produce very low intensity echoes. For example, among the relatively strong echoes are those generated by a muscle-fat interface ($I_r = 0.015 = 1.5\%$), whereas a pure liver-kidney interface would generate much weaker echoes ($I_r = 0.0004 = 0.04\%$) If the angle of incidence with the specular boundary is not 90° , the echo will not travel directly back toward the transducer but rather will be reflected at an angle equal to the angle of incidence (just like visible light reflecting in a mirror).

The potential exists for the echo to miss the transducer and therefore not be detected. If the interface between the tissues is rough, the echo will be

diffusely reflected through a wide range of angles. This decreases the detected echo intensity compared with specular reflection but increases the probability that some echo intensity will be detected by the transducer and displayed in the B mode image. For non perpendicular incidence on an interface between two media with different speeds of sound, the transmitted wave will not continue along the straight-line path of the incident pulse but rather will be deflected by some angle. This deflection is called refraction and is described quantitatively by Snell's law.

If the ultrasound pulse encounters reflectors whose dimensions (d) are smaller than the ultrasound wavelength, scattering occurs. This results in echoes that are reflected through a very wide range of angles. As in the case of diffuse reflection, the detected echo intensity is greatly reduced because it is spread out over such a wide range. However, this ensures that some echo intensity will be detected by the transducer regardless of the angle of the incident pulse. Most biologic tissues appear in US images as though they are filled with tiny scattering structures. The speckle signal that provides the visible texture in organs like the liver is a result of interference between multiple scattered echoes produced within the volume of the incident ultrasound pulse. Most of the signal visible in US images results from scatter interactions.³¹

The appearance of speckle is closely related to the axial and lateral dimensions of the ultrasound pulse. If the lateral pulse width is greater than the

axial pulse length, as is often the case, the speckle “cells” (bright and dark patches of signal) will appear with similar proportions. Varying speckle cell proportions can be observed in regions “a” and “b” of. The orientation of the speckle cells will also reflect the orientation of the ultrasound pulses, which is related to their direction of propagation (ie, the direction of the beam lines). The speckle pattern does not change with time, so successive images obtained through the same plane of the patient with the same transducer position and orientation (ie, beam line directions) will show the same speckle pattern. If the transducer position and orientation are changed, the speckle pattern will generally change as well.

As ultrasound pulses and echoes travel through tissue, their intensity is reduced or attenuated. Attenuation is due to reflection and scattering, which remove intensity from the pulse, from beam divergence and also by friction-like losses. These losses result from the induced oscillatory tissue motion produced by the pulse, which causes conversion of energy from the original mechanical form into heat. This energy loss to localized heating is referred to as absorption and is the most important component of ultrasound attenuation.³¹

Attenuation through tissue is commonly described as loss of intensity in decibels per centimetre of tissue traversed per megahertz. This is because ultrasound attenuation by tissue is approximately proportional to both the total path length and the ultrasound frequency. Longer path lengths and higher frequencies result in greater attenuation.

Ultrasound Pulse Formation³¹

The small ultrasound pulses necessary to implement the imaging approaches discussed earlier are produced by devices called transducers. In general, a transducer is any device that converts energy from one form into another. One example is a light bulb, which converts electrical energy into light and heat. An ultrasound transducer converts between electrical energy and ultrasound waves (mechanical energy). The most important components of ultrasound transducers are piezoelectric elements, which are fabricated of material with the property that applied electrical signals induce mechanical vibrations and applied mechanical vibrations induce electrical signals. This property is called the piezoelectric effect. Composite piezoelectric elements commonly consist of tiny rods of lead zirconate titanate (PZT) ceramic embedded in a matrix of epoxy or similar material.

Ultrasound pulses are formed by applying electrical waveforms to the piezoelectric element, causing it to vibrate and emit ultrasound. Echoes are detected when the reflected echo intensity reaches the piezoelectric element and vibrates it slightly.

These vibrations are converted by the piezoelectric material into electrical signals. The pulses discussed so far for US have been short in duration and in extent. The spatial pulse length (SPL) in the axial direction is equal to the number of cycles in the pulse multiplied by the ultrasound wavelength. Short pulses are desirable since they generally produce US

images with the greatest sharpness in the axial direction (axial resolution). Short pulses are produced by electrically exciting the piezoelectric elements for a very short time, about 1 sec or less. SPL (and axial resolution) remain constant as the pulse propagates to greater depths.

Similarly, ultrasound pulses that are narrow in the lateral direction produce images with greatest sharpness in that direction (lateral resolution).

Narrow ultrasound pulses are produced by focusing the transducer. This may be accomplished for transducers with single piezoelectric elements by curving the element or by using an acoustic lens. These approaches are referred to as mechanical focusing. It is also seen in that all ultrasound beams (focused or not) have widths (and lateral resolutions) that vary with distance from the transducer. The point of narrowest width occurs at the focal distance. For unfocused transducers, this point is also called the near field length. The region closer than this to the transducer is called the near field or Fresnel zone, and the more distant region is called the far field or Fraunhofer zone. Focusing can be accomplished only in the near field of the transducer. For any particular frequency, the mechanical focusing characteristics are fixed as a function of the element curvature or the shape and composition of the lens.

Ultrasound pulses with narrower widths at the focal distance, and thus improved lateral resolution, are achieved by using higher ultrasound frequencies. This produces a trade-off, since the higher frequencies that are

desired for better spatial resolution also produce increased attenuation and thus weaker echo signals at greater depths.

Safety considerations³²

Under certain exposure conditions, ultrasound may cause harmful bio effects. Because of this, there is a hypothetical possibility that ultrasonic imaging may not be completely safe. Consequently, both regulatory authorities and prudent clinicians are concerned to balance

Review the likely benefits of the information which imaging provides against the risk of damage. Although this is the appropriate response to the question of the risk of adverse bio effects due to ultrasonic exposure, the reality is that it is the consequences of misdiagnosis that are likely to be the greatest risk to the patient.

Regulatory agencies in some countries now oblige manufacturers to provide on-screen displays of quantities designated as the ‘thermal index’ (which relates to the risk of causing thermal damage) and the ‘mechanical index’ (which helps primarily to assess the likelihood of damage due to cavitation). These indices are for the guidance of clinicians in assessing risks and benefits and they provide reminders of the importance of applying the ‘as low as reasonably achievable’ (ALARA) principle in the context of justification for individual diagnostic investigations.

Although ultrasonic diagnosis has an impeccable record of safety in relation to the lack of adverse bioeffects, research into thermal, non thermal and other mechanisms of interaction between ultrasound and biological systems continues to be an area of considerable activity. For example, with both the tendency for the ultrasonic exposure levels used by commercially available scanners steadily to be increased in order to obtain more information and the increasing use of microbubble contrast agents, there is fresh anxiety that safety may yet become a real concern.

Ultrasound in Dentistry³³

In diagnostic Ultrasound high frequency sound waves are transmitted in to the body by a transducer and echoes from tissue interface are detected and displayed on a screen. The transducers are designed to produce longitudinal waves hence only those waves can pass through tissues get reflected, Audio frequency of a sound wave is 20 KHz. Anything below this is called infrasonic and above this Ultrasound. Medical Ultrasound uses the frequency of 1-15 MHz (2.5, 3.5, 7.5 and 10 MHz). The transducer has a special property called piezoelectric effect i.e. they can convert sound waves in to electrical waves and vice versa.

Applications

- To view the reduction and healing of fractures.

- To detect parotid lesions, where solid and cystic lesions are reliably differentiated
- To view enlarged cervical lymph node
- Used during FNAC (or) FNAB
- Detection of calculi in major salivary glands
- TMJ imaging
- Detection of Vascular lesions.

Advantages

- No radiation hazards
- Non invasive
- Relatively Inexpensive
- Used for both bone and soft tissue

Looking back over the entire history of medical ultrasonic imaging, it is obvious that the truly major advances which have taken place have resulted from innovations in the applications of physics and engineering.

A REVIEW OF MASSETER MUSCLE BLOOD SUPPLY

The blood flow to the masseter muscle is supplied mainly by 4 groups of arteries. At the superior part of the muscle, blood is supplied by the

branches of the transverse facial artery (BTFAs) superficially and the masseter artery (MsA) medially. The MsA, which arises from the maxillary artery (MxA), runs medially and reaches the medial surface of the muscle near its anterior border. At the mid portion of the muscle, the muscular branch, which arises from the MxA or some- times directly from the external carotid artery (ECA), runs around the posterior border of the ramus of the mandible and across the masseter muscle. From the inferior part, the masseteric branch of the facial artery (MBFA) supplies blood to the muscle. The bifurcation levels of this branch from the main trunk of the FA show relatively wide variations. The branches arising in the inferior part, which are situated near the inferior border of the mandible, run upwards and directly enter into the muscle or sometimes run along the anterior border of the muscle and then enter it.³⁴

A REVIEW OF DOPPLER ULTRASONOGRAPHY³⁵

The Doppler effect produced with ultrasonic frequencies has been used in medicine for almost 50 years. The ability to detect and quantify blood flow by means of Doppler ultrasonography (US) has made this technique an indispensable adjunct to imaging. Although Doppler US has been used clinically for over 20 years, widespread interest in the use of Doppler techniques is a recent development. In the past, use of Doppler US was restricted to evaluation of a few well-defined indications in cardiac disease and assessment of carotid and peripheral arterial and venous disease. The importance of flow information, however, extends well beyond the

identification of stenosis, thrombosis, and occlusion of the major arteries and veins. Doppler US is playing a growing role in the diagnosis of abdominal, pelvic, and fetal disorders.

Production of Ultrasound signal

Conventional B-mode US uses pulse-echo transmission, detection, and display techniques. Brief pulses of ultrasound energy emitted by the transducer are reflected from acoustical interfaces within the body. Precise timing allows determination of the depth from which the echo originates. The gray-scale image derived from these reflected pulses uses only the amplitude information in the returning signal. Rapidly moving targets, such as red cells within the blood stream, produce echoes of such low amplitude that they are not commonly displayed. Fortunately, the backscattered ultrasound beam contains more than the simple amplitude information needed to produce an image. The backscattered signal also varies from the transmitted signal in frequency and phase, and it is this additional information that permits the detection and measurement of target movement.³⁵

The Doppler principle can be understood by considering the interaction of ultrasound with a reflecting interface. The difference between the frequency of the reflected ultrasound and the transmitted frequency is directly proportional to the velocity of the reflecting interface relative to the receiver and is a result of the Doppler effect. The relationship of the returning ultrasound frequency to the velocity of the reflector is described by the

Doppler equation $f_D = (f_R - f_1) = \frac{2f_T v \cos \theta}{c}$ where f_D = Doppler frequency shift, f_R = frequency of sound reflected from the moving target, f_1 = frequency of sound emitted from the transducer, v = velocity of the target toward the transducer, and c = velocity of sound in the medium. The interrelationships of transducer frequency f_1 and the Doppler angle θ to the Doppler frequency shift and target velocity, which are described by the Doppler equation, are of great importance in proper clinical use of Doppler equipment.

Doppler in Signal Processing Several options exist for processing of Doppler frequency shifts to provide useful information about direction and velocity of the moving target (usually red blood cells). Typical Doppler frequency shifts are in the audible range and may be analysed by ear; with training, many flow characteristics may be identified. This approach is, however, subjective and not suitable for quantitative analysis. More commonly, the Doppler shift data are displayed in graphic form as a time-varying plot of the frequency spectrum of the returning signal. A fast Fourier transformation (FFT) is used to perform the frequency analysis. This transformation is important because it permits the Doppler frequency shift information measured by the instrument to be displayed in a fashion that accurately reflects the range of velocities present in the sample being measured. Most Doppler instruments provide a graphic display of Doppler frequencies obtained by means of the FFT at each instant in time against a

time base so that temporal variations in the frequency spectrum can be studied.³⁵

The resulting Doppler frequency spectrum displays

- a) The variation with time of the Doppler frequencies present in the volume sampled
- b) The envelope of the spectrum, which represents the maximum frequencies present at a given point in time
- c) The width of the spectrum at any point, which indicates the range of frequencies present. In many instruments, the amplitude of each frequency component is displayed in grey scale.

In colour Doppler imaging systems, velocity information determined from Doppler measurements is displayed as a feature of the image itself. In addition to the detection of Doppler frequency shift data from each pixel in the image, these systems may also provide range-gated pulsed Doppler with spectral analysis for display of Doppler data.

The clinical applications of Doppler US include

- identification of vessels
- determination of the direction of blood flow
- evaluation of narrowing or occlusion
- Characterization of flow to organs and tumors.

Analysis of the Doppler frequency shift as it varies with time (ie, in the cardiac cycle) can also be used to infer both proximal stenosis and changes in distal vascular impedance.

Most radiologists who have used pulsed Doppler US have emphasized the detection of stenosis, thrombosis, and flow disturbances in major peripheral arteries and veins. In these applications, measurements of peak systolic and end diastolic frequency on velocity, analysis of the Doppler spectrum, and calculation of certain frequency or velocity ratios have been the basis of analysis.

More recently, the use of Doppler US in the inference of abnormalities in the peripheral vascular bed of an organ on tissue has gained attention. Changes in the spectral waveform, which are measured in terms of indexes that compare flow during systole and diastole, provide insight into the resistance of the vascular bed supplied by the vessel and indicate changes caused by a variety of pathologic conditions. Changes in these indexes, compared with normal values, may be important in the early identification of rejection of transplanted organs, parenchymal dysfunction, and fetal compromise due to intrauterine growth retardation. Also, characteristic Doppler signals have been described that may eventually aid in differentiation of benign from malignant masses.³⁵

REVIEW OF ULTRASONOGRAPHY OF MASSETER MUSCLE

Intensive use of any skeletal muscle may cause changes in the muscle fibre size and composition, which in turn will increase the strength of the muscle and the resistance to fatigue. This is also true of the masticatory muscles. Prolonged high activity of these muscles resulted in increased ultrasonographic thickness of the masseter muscle.

Morse MH et al (1990)³⁶ conducted a study to confirm the suitability of ultrasound in diagnosis, and to establish a normal range. Sixty-two masseter muscles were measured using a standardized technique and the derived normal range for transverse dimension was 8.5-13.5 mm. Three instances of clinically diagnosed masseteric hypertrophy were examined; in each case measured transverse masseteric dimension was significantly greater than the normal range. The study concluded that direct ultrasonic measurement of masseteric bulk should replace computed tomography as the definitive investigation.

Kiliaridis S, Kålebo P (1991)³⁷ conducted a study to evaluate ultrasonography as a method for measuring masseter muscle thickness, to quantitate the normal range of the ultrasonically measured thickness of the masseter in adults, and to test whether the variation in the thickness of the muscle is related to the variation in the facial morphology in different individuals. In 40 healthy, fully-dentate young adults, 20 men and 20 women, the masseter thickness was measured bilaterally by a real-time ultrasound imaging technique. The measurements were performed under both relaxed

conditions and with maximal clenching. Standardized facial photos of the subjects were taken so that their facial morphology could be determined. Under relaxed conditions, the mean thickness of the muscle in men was 9.7 (+/- 1.5) mm, and under contracted conditions, 15.1 (+/- 1.9) mm. In women, the respective measurements were 8.7 (+/- 1.6) mm and 13.0 (+/- 1.8) mm. The thickness of the masseter muscle was found to be related to the facial morphology, mainly in women, but not in men; the women with a thin masseter had a proportionally longer face. Ultrasonography was found to be a reliable and accurate method for study of the thickness of the masseter muscle. There was a large variation in the thickness of the muscle between individuals, and the thickness of the masseter was related to facial morphology in women

Bakke M et al.(1992)³⁸ measured the thickness of the human masseter muscle, corresponding approximately to a cross-section at the most bulky part of the superficial portion, by ultrasound scanning at three sites 1 cm apart. The study included 13 subjects, 21-28 years of age, with a minimum of 24 teeth and without craniomandibular disorders. Ultrasonography produced a well defined depiction of the muscle with distinct tendinous structures. The average thickness at the measuring sites varied from 8.83 to 11.08 mm with the muscle relaxed, and increased significantly during contraction to average values between 9.84 and 12.57 mm. The study showed a connection between measures of masseter thickness and function of the muscle, as well as parameters generally associated with masseter muscle function. Muscle

thickness at the voluminous anterior part of the superficial portion was systematically and significantly correlated to bite force, occlusal tooth contact and cephalometric data (anterior face height, vertical jaw relation and mandibular inclination). In conclusion, ultrasound scanning gave an uncomplicated and a reproducible access to parameters of jaw muscle function and its interaction with the craniomandibular system

Ariji et al. (1994)³⁹ studied ultrasonographic images of 32 Japanese patients with inflammatory change in the masseteric region to clarify the characteristic findings and to evaluate the utility of ultrasonography. Inflamed masseter muscles frequently demonstrated reduction of echo intensity and complete or partial absence of hyperechoic bands. The mean thickness of the masseter muscle on the unaffected side was 8.6 mm, whereas that on the affected side was 12.9 mm.

Raadsheer et al (1994)⁴⁰ Non-invasive imaging techniques such as computerized tomography, magnetic resonance imaging (MRI), and ultrasonography enable measurements of the cross-section and thickness of human jaw muscles in vivo, providing an indication of the maximal force a muscle can exert. In 15 adult Caucasian men the thickness of the masseter muscle was registered bilaterally on three different levels by ultrasonography. Scans were made on the contracted and relaxed muscle. A comparison was then made with measurements from serial MRI scans, using univariate analysis of variance for repeated measurements and Pearson's correlation

coefficients. They concluded that ultrasonography is an accurate and reproducible method for measuring the thickness of the masseter in vivo

P.J. CLOSE et al (1995)⁴¹ conducted a study to determine the relationship between linear dimensions of human masseter muscle cross-section and cross-sectional area (CSA), and to assess symmetry between the two sides in normal young adults. Cross-sectional images of the masseter muscle were measured bilaterally by real-time ultrasound imaging in 39 healthy dentate subjects, 19 males and 20 females, aged 21-47. From stored images, CSA and two linear measurements of muscle cross-section were obtained (the shortest and the longest distance through the muscle group). Correlation and regression analyses were performed to examine the relationship between CSA and the linear dimensions (both individually and with the linear dimensions multiplied). Symmetry of CSA between the two sides of the face was examined using the paired t-test. The significance of correlation coefficients (r) and the difference between the slopes of the regression lines were also examined. Masseter CSA was larger in males than in females. All correlation values between CSA and linear measurements were significant. Although the correlation in this regard was high, the linear dimensions consistently overestimated the actual CSA by approximately 25%. Males showed more symmetry of CSA than females.

EMSHOFF AND BERTRAM et al (1995)⁴² carried out anatomic assessment with high resolution grey scale ultrasonography in the evaluation

of the masticatory muscles of patients with TMJ disorders who had symptoms of muscle pain and tenderness on palpation. Thirty-seven women and 13 men with a mean age of 26 years fulfilled the following criteria for selection: (1) signs and symptoms of mandibular dysfunction verified clinically, (2) unilateral muscle pain or muscle tenderness on palpation. The results of this study show that B-scan sonography is an inexpensive and non-invasive diagnostic technique with relatively high sensitivity and specificity that should be used to supplement clinical examinations in patients with TMJ disorders. It can be used to locate affected muscles that do not have the symptom of tenderness on palpation and thereby aid treatment planning.

Kiliaridis S et al.(1995)⁴³ performed Ultrasound technique to measure the thickness and examine the internal structure of the masseter muscle in a group of 16 adult patients (nine women and seven men) with myotonic dystrophy (MyD) and 16 healthy individuals matched in age, sex and number of occluding teeth. The masseter thickness was measured bilaterally under both relaxed conditions and during maximal clenching. The imaging characteristics of the masseter in most of the MyD patients was an obvious atrophy of the muscle with increased echo intensity of the intramuscular tissue and loss of the internal structure concerning tendons and fasciae. The mean masseter thickness in the MyD group was 10.4 (+/- 2.2) mm under relaxed conditions and 11.1 (+/- 2.4) mm during maximal clenching, compared with

13.3 (+/- 2.2) mm and 14.1 (+/- 2.4) mm, respectively, in the healthy group ($P < 0.001$).

Bakke et al. (1996)⁴⁴ evaluated the effect of static and dynamic activity from changes in masseter thickness as a measure of oedema, simultaneously with assessment of perceived pain/discomfort and cardiovascular responses. As static activity, fourteen young healthy women bit at 15% maximal voluntary contraction on bite-force transducers in the molar regions until exhaustion or 20 min at maximum (median endurance time 7.1 min). For dynamic activity, the same individuals chewed gum unilaterally until exhaustion or 40 min at maximum (all endured 40 min) with a cycle time of 725 ms, an average load of 9.3% of maximal electromyographic activity (max EMG) and a peak mean voltage of 54.3% of max EMG. Muscle thickness was measured by ultrasonography at the mid-portion of the ipsilateral masseter. Immediately after exercise, muscle thickness was significantly increased, more after static (14.0%) than dynamic (8.6%), and returned to pre-exercise values after 20 min recovery. Visual analogue scales (VAS) revealed the concomitant occurrence of pain (static 11.9 VAS%; dynamic 5.9 VAS%) and discomfort (static 8.1 VAS%; dynamic 5.9 VAS%), and both sensations decreased to pre-exercise values after 20-min recovery. Systolic blood pressure increased significantly, more during static (12.5%) than dynamic activity (4.3%), whereas heart rate rose significantly only during dynamic exercise (13.3%). With the individual under investigation sitting

upright, scanning was done on the most convenient side, corresponding to a cross-section of the superficial portion of the muscle. The thickness was measured with the masseter relaxed (the teeth close to the intercuspal position) and during maximal intercuspal clenching. Each time the thickness was assessed as the average of three measurements. Hence, activity was associated with muscular swelling and pain, and, despite the relatively small size of the masticatory muscles, also with general cardiovascular responses.

M KUBOTA et al (1998)⁴⁵ conducted a study to investigate the Masseter muscle thickness to relate with maxillofacial morphology in 80 adult healthy male volunteers with a mean age of 23 years. The masseter muscle thickness was measured using ultrasonography in which relaxed muscle thickness was 15.3 mm with standard deviation of 3 and contracted muscle thickness was 14.2 with standard deviation of 2.7. They concluded that masticatory function influences morphology of mandible.

P C M BENNINGTON et al (1999)⁴⁶ measured volume, cross section area, thickness, width and length of contracted masseter muscle in a sample of 10 adults 4 males and 6 females using three dimensional ultrasonography. The scans were carried out bilaterally using hand held probe with mechanical position sensors that enabled the computer to reconstruct the images using a 3D array of slices. The mean value obtained was 11.1 mm in Males with a standard deviation of 1.3 and 9.5 mm in Females with standard deviation of 1.2.

S. Bertram et al (2003)⁴⁷ conducted a study to determine whether the scanning level may affect cross-sectional measurements and whether measurements made at identical levels may be reproducible. The study included 42 asymptomatic volunteers. Unilateral ultrasonographic investigation was performed with a linear (B-scan) 7.5 MHz small-part transducer to register cross-sections of the masseter muscle on five different levels. Scans were made on the relaxed and contracted muscle. Measurements were made in two sessions with a time interval of at least 5 min.

For the ultrasonographic measurements the main effects of the variables 'session' ($P=0.0001$), 'level' ($P=0.0001$), and 'condition' ($P=0.0001$) were significant. The study concluded that Cross-sections evaluated at the middle (method error=0.31 mm; error=2.0%) and lower level (method error=0.32 mm; measurement error=2.4%) of the contracted muscle were the most reproducible. The conclusion is that ultrasonography is a reproducible method for measuring cross-sections at the middle and lower level of the contracted masseter muscle.

S. KILIARIDIS et al (2003)⁴⁸ carried out a study to determine the relationship between the ultrasonographic thickness of the masseter muscle and the width of the maxillary dental arch. The sample comprised 60 consecutive orthodontic patients (37 females, 23 males), 7–18 years of age with a Class I relationship and minor malocclusion. The thickness of the masseter muscle was measured ultrasonographically. Recordings were

performed bilaterally with the muscles both in relaxation and under contraction. The mean masseter muscle thickness was 11.6 mm (SD = 1.4) in relaxation and 11.9 mm (SD = 1.6) during contraction in the female group versus 12.1 mm (SD = 2.2) in relaxation and 12.4 mm (SD = 2.2) during contraction in the male group. The study concluded that the masseter muscle was thicker in older individuals and in males.

K. Kubo et al. (2006)⁴⁹ measured Masseter muscle thickness to elucidate the outer shape changes of the whole masseter with clenching by multipoint measurement of serial ultrasound images. Serial coronal images (perpendicular to the FH plane, 3 mm interval) of the right masseter of five healthy men were obtained with a real-time ultrasonography equipped with a 13 MHz linear-array transducer. Registrations were made during muscle relaxation and maximal clenching. The distance between the lateral and medial outlines of the muscle was measured at intervals of 1 mm from the origin to insertion in each image as the thickness at the corresponding measured point. The thickness of the relaxed and contracted muscle (R and C) and its difference (D) varied among the measured points. The mean muscle thicknesses at R-max point and at C-max point in five subjects were 12.8 with SD of 1.2 and 15.7 with SD of 1.1 mm, respectively. Muscle thickness at most measured sites increased with clenching, whereas it decreased at several sites, mainly near the origin and insertion. There were positive correlations in every subject both between R and C. Changes of thickness with clenching showed

great disparity within the masseter, which may result from the complexity of the contraction properties due to the multipennate structure and functional heterogeneity.

Rozylo-Kalinowska et al (2006)⁵⁰ studied ultrasonography of masseter muscle to determine normal ranges of masseter muscles dimensions and to evaluate the role of ultrasonography in imaging algorithm of masseter pathologies.

The material consisted of 48 adults aged 20–26 without signs and symptoms of masseteric pathologies. Moreover 7 patients were examined aged 16–42 referred for ultrasonography due to asymmetric or symmetric swelling of mandibular angle region, in whom salivary pathology was suspected and not confirmed by ultrasonographic findings. Normal masseters were examined by means of a 7MHz linear ultrasound probe. Their thickness was measured in horizontal plane parallel to inferior mandibular border in three lines as well as in vertical plane parallel to mandibular ramus on three levels. Medical history concerning pathological mastication (e.g. gum chewing, bruxism) was recorded and compared with the dimensions of the examined muscles. It was found that ultrasonography was a valuable diagnostic aid in detecting pathologies within masseter muscles. The ranges of dimensions of normal masseter muscles are very broad and in clinical practice it is essential to detect asymmetry of the right and left muscles, as well as enlargement over 19 mm.

Stavros Kiliaridisa et al (2007)⁵¹ studied three groups of growing individuals to assess the bilateral differences in the thickness of the masseter muscles. Three groups of growing individuals were studied: (1) untreated group: 38 individuals with unilateral crossbite, (2) control group: 224 subjects without transversal malocclusions (3) treated group with quad helix. In the untreated group, the thickness of the masseter muscle on the crossbite side was statistically significantly thinner than the one on the normal side ($P .025$). No statistically significant differences were found in the thickness of the masseter muscle between the left and the right side in the control group. In the treated group, no statistically significant differences were found in the thickness of the masseter muscle between the former crossbite side and the normal one.

L. J. PEREIRA et al (2007)⁵² used ultrasonography to determine the association between muscle thickness and bite force. The study was carried out in 40 adolescents 12-14 yrs. of age and bite force measured with a pressure transducer. The study showed positive correlation between masseter and bite force.

Hyoung-Zoo Hwang et al (2008)⁵³ conducted a study to assess the internal echo intensity and morphological variability of masseter muscles on ultrasonography and to establish diagnostic criterion of estimation. Participants consisted of 50 young adults (male 25, female 25) without pathologic conditions and with full natural dentitions. Sonographic examinations were done with real time ultrasound equipment as Logiq 500

(GE Medical Systems, Seoul, Korea) at 3 parts according to lines paralleling with ala-tragus line as reference line. The thickness and area of masseter muscles according to reference line in cross-sectional images were measured at rest and at maximum contraction. The Relaxed and contracted Muscle lengths obtained were 12.6 mm with standard deviation of 2.6 and 16.6 mm with standard deviation of 1.3 respectively. When comparing the thickness and area of masseter muscles concerning with gender, there was few significant difference between right and left sides, however, there were significant differences between males and females except for the greatest thickness of left side. The changes of the greatest thickness and the area between rest and maximum contraction showed that the part of the least thickness manifested more increase at maximum contraction. Each part the manifestations of the internal echogenic intensity of the masseter muscles were different depending on the locations. But there was no statistically significance differences. The study concluded that Changes of muscles thickness with contraction and internal echogenic intensity with locations showed great disparity within the masseter muscles, which will be diagnostic criteria for pathophysiologic and anatomic changes of Masseter muscle.

Rajeshwari G et al (2010)⁵⁴ conducted a study to measure thickness of masseter muscle at rest and at maximum clenching position by ultrasonography with masseter muscle hypertrophy in oral submucous fibrosis patient and control group, and also to establish the normal value of masseter

muscle thickness ultrasonographically and to prove that ultrasonography is reliable diagnostic technique for the evaluation of masseter muscle hypertrophy in oral sub mucous fibrosis patient.

Ultrasonographic measurements (3-12) Mhz of Masseter muscle thickness were performed for 40 male subjects comprising 20 oral sub mucous fibrosis patients and 20 normal patients. Ultrasound examinations of Masseter muscle was done by a line drawn on the skin parallel to and 2 centimeters above the inferior border of mandible approximately corresponding to the most bulky part of superficial portion of masseter muscle. Ultrasound examination was performed with patient in supine position by single examiner using Philips CDH 100 ultrasound imaging system. The imaging was performed bilaterally in both relaxed and contracted states. The ultrasound scans from three measuring sites constitute a full representation of cross section of superficial portion of Masseter muscle. The average of three measurements were recorded in millimetres.

In the control group, the mean relaxed thickness of Right and Left masseter muscle was 7.66 +/- 0.87 mm and 7.84 +/- 0.71 mm respectively, whereas the mean contracted thickness of right and left masseter muscle was 9.98 +/- 0.87 mm and 10.33 +/- 0.51 mm respectively. In the study group the mean relaxed thickness of right and left masseter muscle was 9.42 +/- 1.24 mm and 9.55 +/- 1.16 mm respectively, whereas the mean contracted thickness of right and left masseter muscle was 13.51 +/- 1.86 mm and 12.99

+/- 1.75 mm, respectively. The comparison of right versus left masseter muscle thickness was not significant in control group both in relaxed and contracted state. The study group showed higher thickness both on right and left side muscle and also in relaxed and contracted state when compared to controls. The thickness of muscle is more in contracted state than in relaxed state which was highly significant. The results showed that the study group showed higher thickness both on right and left side muscle and also in relaxed and contracted state when compared to controls. The thickness of muscle is more in contracted state than in relaxed state which was highly significant.

Sushma and Ravi (2010)⁵⁵ measured thickness of masseter muscle with ultrasonography to study association between muscle thickness and facial morphology. 72 individuals between age of 18 and 25 years were studied. Relaxed state values were 10.429 with a standard deviation of 1.118 and contracted state values were 12.838 with standard deviation of 1.326. They concluded that strong association exists between masseter muscle and facial morphology.

P.S. Bhoyar et al (2012)⁵⁶ conducted a study to determine the changes in masseter muscle thickness due to the state of complete edentulism and the effect of complete denture rehabilitation on the masseter muscle. Real-time ultrasonography of the masseter muscle at relaxed and contracted states was carried out for twelve patients (six dentulous and six completely edentulous). The results obtained in dentulous patients in Relaxed state were

Left- 15.5 mm with standard deviation of 0.11 and Right- 15.1 mm with a standard deviation of 0.09. The Contracted state thickness were Left- 18.6 mm with a standard deviation of 0.10 and Right- 17.7 mm with standard deviation of 0.13. Thus they concluded that the thickness of the muscle in edentulous condition remains smaller than that of dentate individuals.

Ariji et al (2001)⁵⁷ conducted a study to clarify the normal findings of arteries in and around the masseter muscle and to present their pathologic changes with the use of color Doppler sonography. The vascular appearances were examined for the arteries feeding the masseter muscle in healthy volunteers (n = 38) and patients with inflammation (n = 5) and intramuscular hemangioma (n = 3). The features of these arteries were investigated together with the flow velocities and arterial resistances. The symmetry indices were also calculated to assess the pathologic changes. The vascular appearances were examined for the main arteries feeding the masseter muscle in healthy volunteers (n = 38) and patients with inflammation (n = 5) and intramuscular hemangioma (n = 3). The detection rates of the branch from the transverse facial artery was 98.7% in healthy volunteer. Examination was performed with the use of a Logiq 700 (GE Yokogawa Medical Systems, Tokyo, Japan) equipped with a 12 MHz-wide bandwidth linear active matrix transducer (range, 6-14 MHz). The sonogram was obtained as follows: a focal range was set as multi focus from 0.5 to 2.0 cm; an image depth was set as 4 cm. Color Doppler sonography was performed under these settings to optimize the blood

flow in the target artery. Wall filter and pulse-repetition frequency were adjusted to avoid arising artifact. The Doppler angles were adjusted according to the direction of blood flow, and the sample volume sizes were set at 2 to 3 mm. The color gain was set at 38 decibel.

Scanning directions of these arteries were determined with reference to the anterior border of the masseter muscle. Ultrasound beams were set in the direction perpendicular to the surface of the underlying ramus of the mandible in all examinations.

The BTFA was scanned parallel to the anterior border of the muscle at the posterior third of the muscle width. They concluded that Color Doppler sonography is useful in describing the arteries in and around the masseter muscle and has the potential of being used to depict the pathologic changes.

Study Topic

Comparison of Masseter muscle changes in Tobacco chewers and non-chewers using Ultrasonography.

Study Design

This is a Case Control type of study.

Study Duration

This study was conducted between March 2012 and June 2012 by Department of Oral Medicine, Diagnosis and Radiology at Ragas Dental College and Hospital and Bharat Scans private limited, Chennai.

STUDY POPULATION

A total number of 40 patients were involved in the study

OBTAINING APPROVAL FROM THE AUTHORITIES:

Permission from the Institutional Review Board of **Ragas Dental College and Hospital, Chennai** and Bharat Scans Private limited, Royapettah, Chennai was obtained before starting the study.

Due consent to participate in the study was obtained from the Subjects in letter format both in Tamil and English.

MATERIALS

Examination of Patients

- Dental chair with halogen lamp
- Sterile Kidney tray
- Mouth mirror
- Dental Explorer
- Disposable mouth mask
- A pair of sterile gloves

Equipment

- Sonic Gel
- Linear Transducer
- Monitor
- Printer

METHODOLOGY

The study comprised of a total number of 40 male patients. Out of the 40 patients, 20 were non chewers and the other 20 were tobacco chewers.

CONTROL GROUP

The Control group comprised of 20 male patients comprising of ten in the age group of 21- 30 yrs. and ten in age group of 31- 40 yrs. with no tobacco chewing habit visiting the Out Patient Department of Oral Medicine, Diagnosis and Radiology at Ragas Dental College and Hospital, Chennai.

Inclusion Criteria:

- Patients with no tobacco chewing habit in any form
- Patients with full natural dentition

Exclusion Criteria:

- Participants with Gum chewing habit, Temporomandibular disorders, Bruxism and Malocclusion
- Patients with Pulpoperiodontal pathology
- Patients with any mucosal lesions
- Female patients

STUDY GROUP

The study group comprised of 20 male patients comprising of ten in the age group of 21- 30 yrs. and ten in age group of 31- 40 yrs. with tobacco chewing habit visiting the Out Patient Department of Oral Medicine, Diagnosis and Radiology at Ragas Dental College and Hospital, Chennai.

Inclusion Criteria

- Individuals who chew tobacco in packets
- Frequency of more than 2 packets per day
- Duration > 2 years
- Patients with full natural dentition

Exclusion Criteria:

- Participants with Gum chewing habit, Temporomandibular disorders, Bruxism and Malocclusion
- Patients with Pulpoperiodontal pathology
- Patients with any mucosal lesions
- Female patients

INFORMED CONSENT:

Permission from the Institutional Review Board of **Ragas Dental College and Hospital, Chennai** and Bharat Scans Private limited, Royapettah, **Chennai** was obtained before starting the study.

Consent was prepared in both Tamil and English. Informed consent was taken from all subjects before including them in the study.

EXAMINATION OF THE SUBJECTS

The experimental subjects were made to sit comfortably on a dental chair and examined under halogen lamp. Relevant demographic data was collected. An Intra Oral examination is carried out. The findings were documented in the proforma.

ULTRASOUND EXAMINATION^{45,47}

The purpose of study is explained to the patient and consent is obtained.

Ultrasonographic examination of masseter muscle is performed for patients in Control and study Group.

Examinations were Performed using Linear 11 MHz small part Transducer connected to Voluson E8 sonograph (GE medical systems Inc., USA). With the measurements made directly on the screen at the time of scanning, recorded to the nearest 0.1 mm. The sonograms were performed by a single experienced sonologist. All trials were conducted in a darkened room with the patient sitting in an upright position and the Frankfort Horizontal plane parallel to the floor. The orientation of the scan series was based on a standardized protocol to obtain cross-sections intersecting the muscles perpendicular to their long axis. The origin, insertion and anterior border of

the masseter were determined by palpation. The scanning levels were orientated parallel to the occlusal plane.

The thickness of masseter muscle was measured at a site on the skin of cheek where a line joining the lateral commissure of the mouth to the intertragic notch of the ear crossed the masseter muscle, the thickest part of the masseter, close and approximately parallel to occlusal plane. The images of muscle during relaxation and contraction were recorded bilaterally and copied on to the disc. The transducer was held against the cheek with light pressure and oriented perpendicular to the cortex of the underlying ramus. The transducer was tilted to be perpendicular to the ramus until the ramus was depicted on the screen as a sharp white line. Scans were made on the relaxed and contracted muscle. Relaxation was obtained by asking the subject to maintain slight interocclusal contacts, contraction by asking him to clench. The masseter muscle is identified on sonograms as a homogenous structure adjacent to echogenic band of mandible.

Cross sections are studied. The registrations are performed three times with a time interval between the two measurements of about 3 min and the results are obtained from the mean of the measurements. The thicknesses of the muscles are measured directly on the screen of the scanner. The measurements obtained were recorded in millimetres.

Doppler Examination⁵⁷

The settings are then changed to Doppler mode to detect branch of transverse facial artery of Right and Left Masseter muscle. Examinations were Performed using Linear 11 MHz small part Transducer connected to Voluson E8 sonograph (GE medical systems Inc., USA) The sonogram was obtained as follows: a focal range was set as multi focus from 0.5 to 1.5 cm; an image depth was set as 2.5 cm. Color Doppler sonography was performed under these settings to optimize the blood flow in the target artery. The Doppler angles were adjusted according to the direction of blood flow. Scanning directions of the artery was determined with reference to the anterior border of the masseter muscle.⁵⁷

Ultrasound beams were set in the direction perpendicular to the surface of the underlying ramus of the mandible during examinations. The branch of transverse facial artery was scanned parallel to the anterior border of the muscle at the posterior third.

Detection rate was defined as the percentage of the number of subjects with visible artery examined on color Doppler images.

STATISTICAL ANALYSIS

All the data were entered in Microsoft excel sheets. Statistical analysis was done using SPSS software.

Mean: defined as sum of values (X) divided by the number of values (N) and denoted by.

$$\text{Mean (X)} = \frac{\sum X}{N}$$

Chi Square Test

$$X^2 = \frac{\text{sum of (observed frequency - expected frequency)}^2}{\text{Expected frequency}} = \frac{\sum (O-E)^2}{E}$$

t test for difference of two mean

$$t = \frac{|\bar{X}_1 - \bar{X}_2|}{S \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \sim t_{n_1+n_2-2 \text{ df}}$$

where
$$S = \sqrt{\frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{n_1 + n_2 - 2}}$$

S is combined standard deviation

\bar{X}_1 is mean for group I

\bar{X}_2 is mean for group II

n_1 is sample size in group I

n_2 is sample size in group II

$P > 0.05$ = Difference is not significant

$P \leq 0.05$ = Difference is significant (S)

$P \leq 0.01$ = Difference is highly significant (S)

$P \leq 0.001$ = Difference is very highly significant (HS)



Fig.1: Armamentarium for clinical examination



Fig.2: Voluson E8 sonograph (GE medical systems Inc., USA)



Fig.3: 11 MHz linear transducer probe



Fig.4: Sonic gel



Fig.5: Measurement of masseter muscle length on the skin of cheek at a line joining the lateral commissure of the mouth to the intertragic notch of the ear



Fig.6: Measurement of length of Masseter muscle at Relaxed and Contracted state

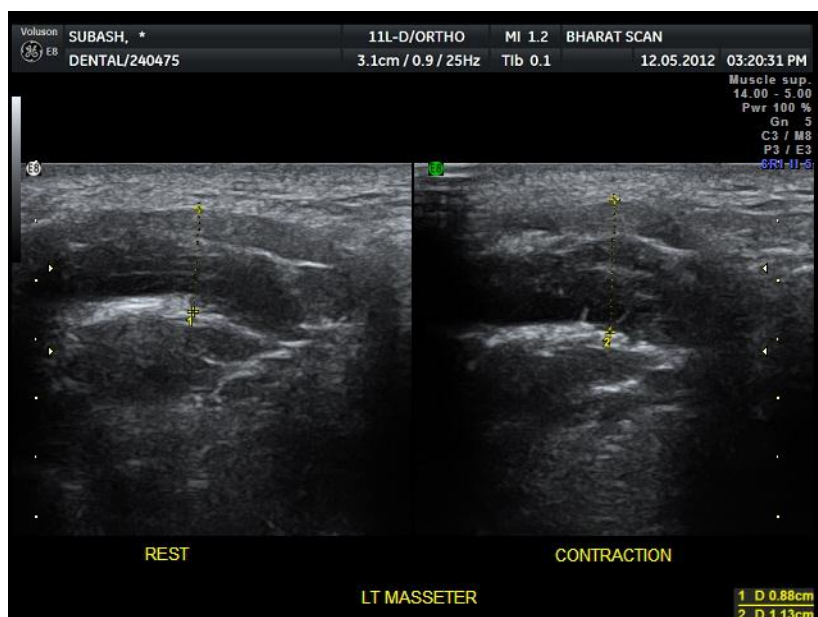


Fig.7: Ultrasonograph showing Left side Relaxed and Contracted muscle lengths in a Control group subject

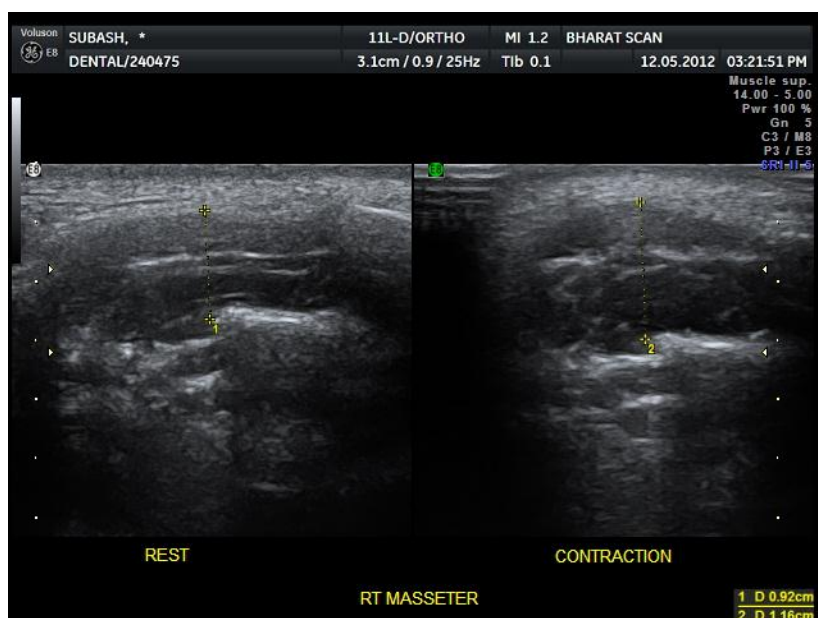


Fig.8: Ultrasonograph showing Right side Relaxed and Contracted muscle lengths in a Control group subject

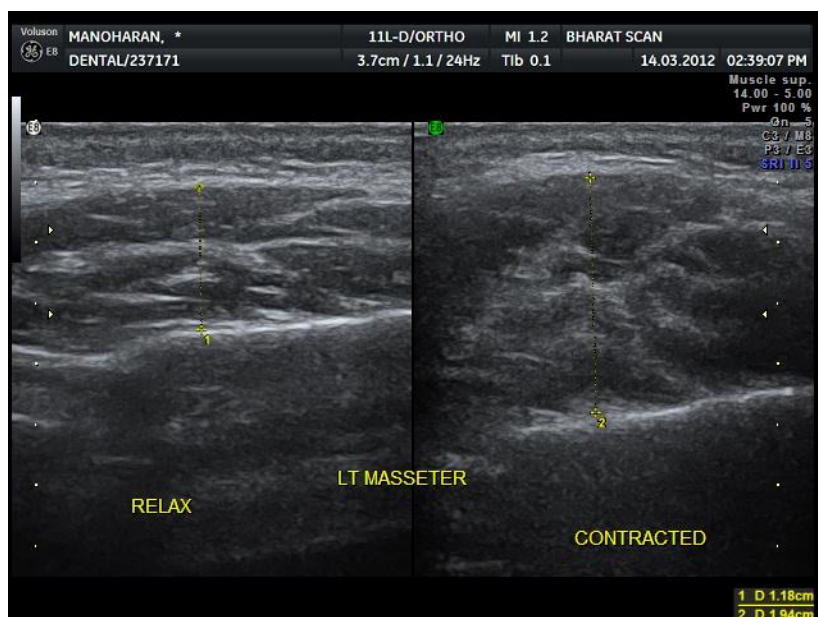


Fig.9: Ultrasonograph showing Left side Relaxed and Contracted muscle lengths in a Study group subject

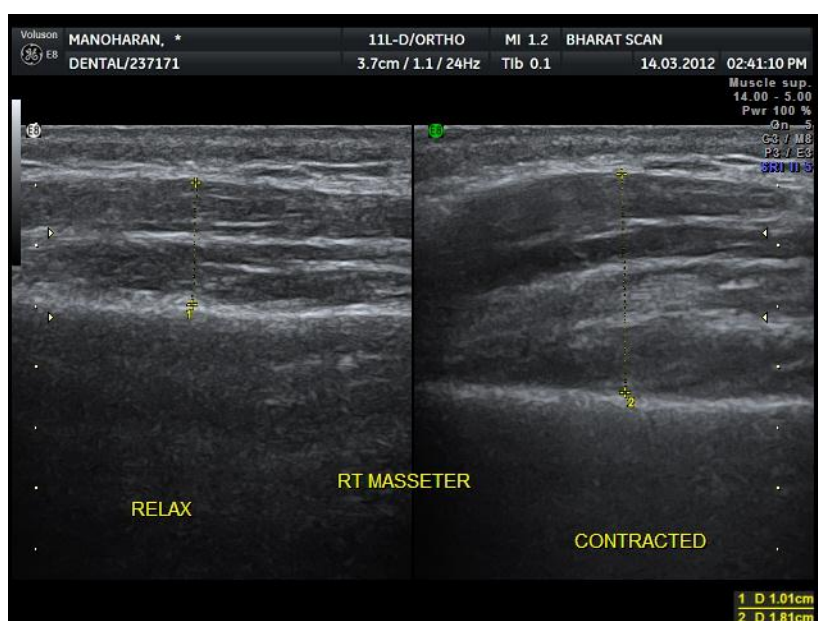


Fig.10: Ultrasonograph showing Right side Relaxed and Contracted muscle lengths in a Study group subject



Fig.11: Left side Doppler ultrasonograph in a Control group subject with artery detected



Fig.12: Left side Doppler ultrasonograph in a Control group subject with artery not detected

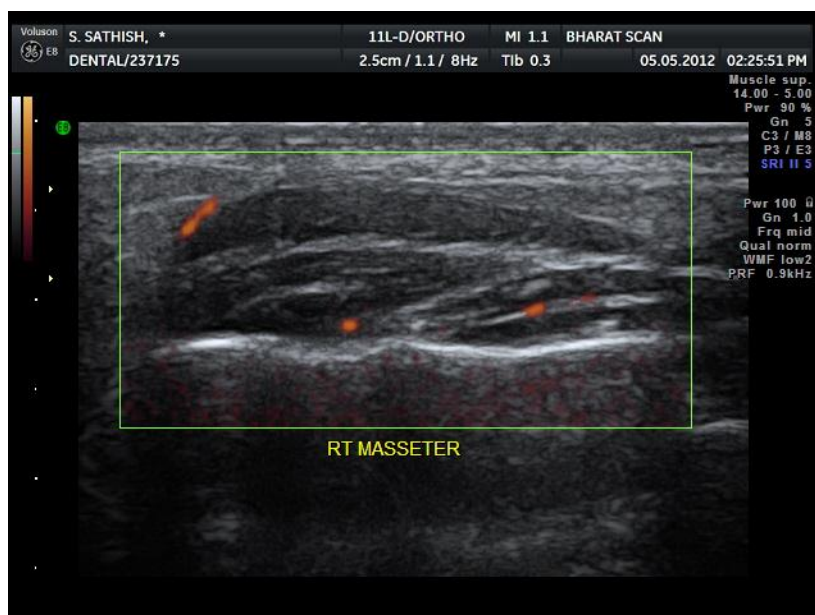


Fig.13: Right side Doppler ultrasonograph in a Control group subject with artery detected

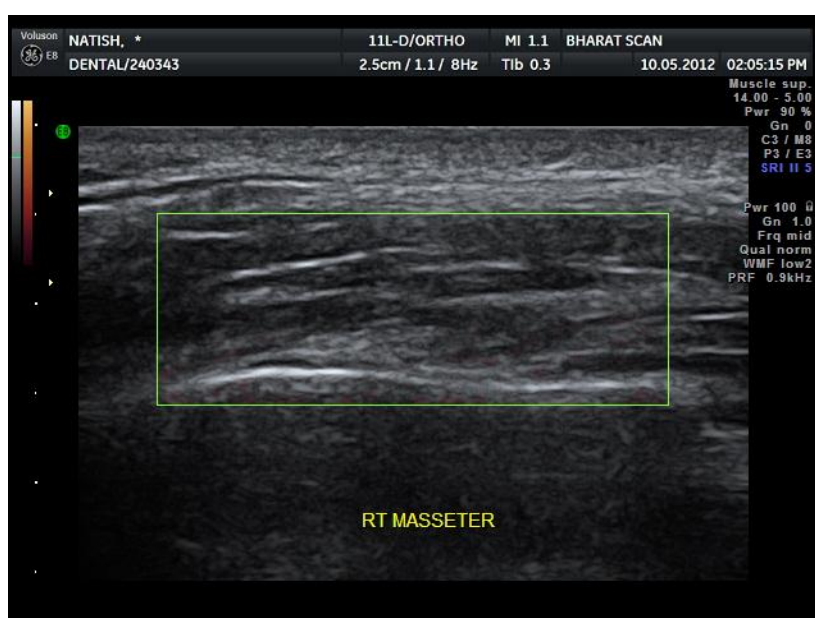


Fig.14: Right side Doppler ultrasonograph in a Control group subject with artery not detected

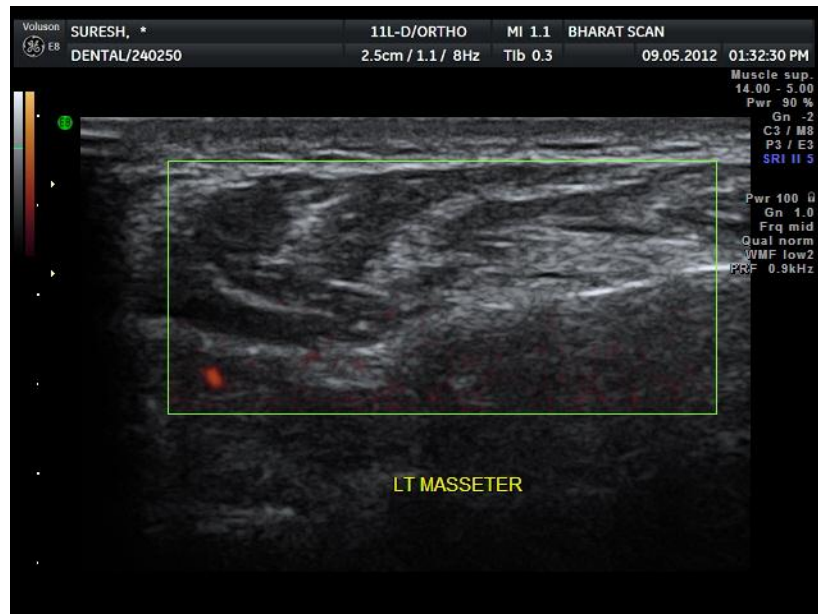


Fig.15: Left side Doppler ultrasonograph in a Study group subject with artery detected

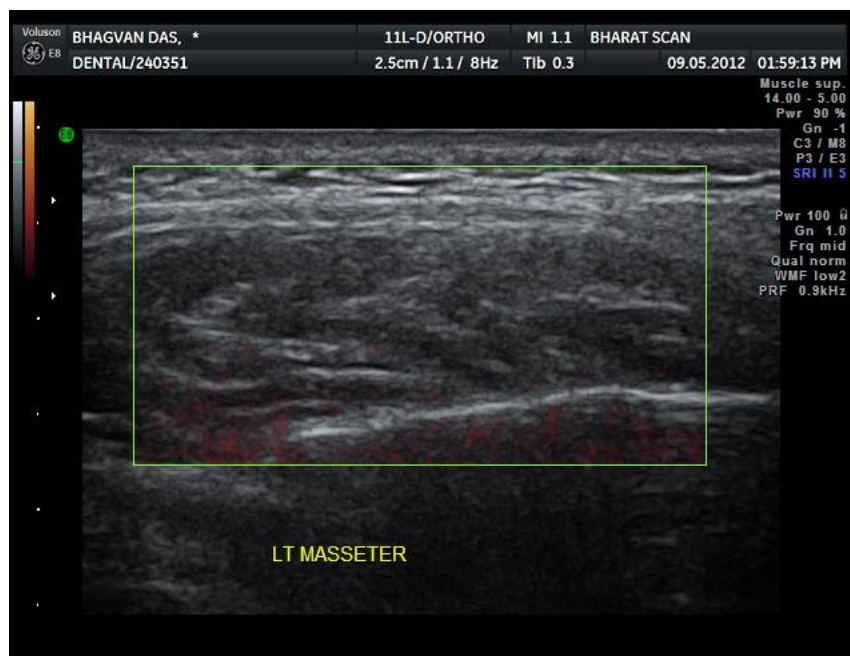


Fig.16: Left side Doppler ultrasonograph in a Study group subject with artery not detected

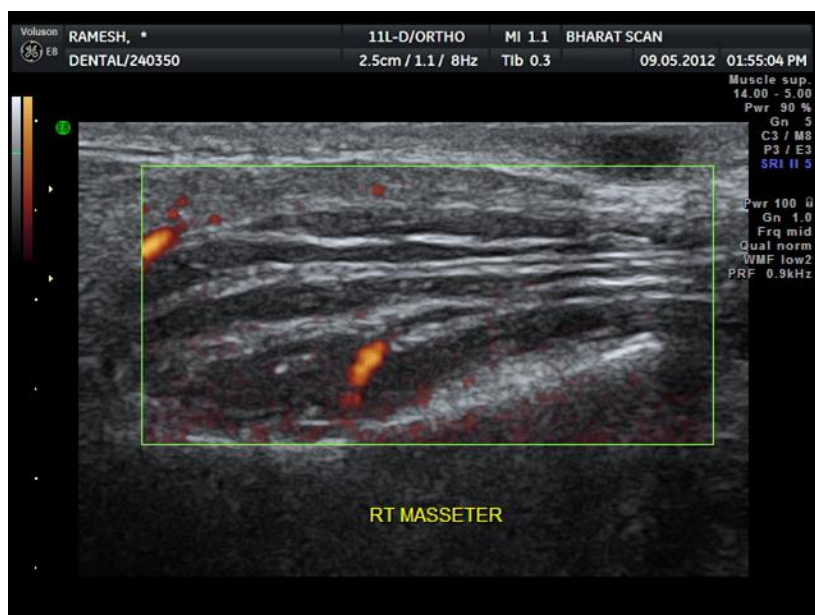


Fig.17: Right side Doppler ultrasonograph in a Study group subject with artery detected



Fig.18: Right side Doppler ultrasonograph in a Study group subject with artery not detected

The present study is a Case Control study conducted by Department of Oral Medicine, Diagnosis and Radiology at Ragas Dental College and Hospital, Chennai at Bharat Scans Private limited Royapettah, Chennai. It was devised to compare the Masseter muscle changes in Tobacco chewers and non chewers through Ultrasonographic measurement of masseter length and to detect branch of transverse facial artery in the Masseter muscle through Doppler ultrasound. The study was conducted between March 2012 and June 2012 with 20 Tobacco chewers and 20 Healthy non chewers. The data obtained from the study was statistically analysed. The results extracted were compared with various variables included in the study and are presented here.

Table 1: Age comparison of subjects in control and study groups

This table shows comparison of age group in years in control and study groups. In control group of 20 subjects the mean age obtained was 28.55 years with a standard deviation of 6.16 and in study group of 20 subjects the mean age obtained was 32 years with a standard deviation of 5.35. The p value obtained was 0.074 which is > 0.05 and is insignificant.

Table 2: Relaxed Right Masseter and Contracted Right Masseter measurements in Control group

This table denotes Relaxed and Contracted muscle lengths of Right side Masseter muscle of Control group. The mean Relaxed Right Masseter length obtained was 8.70 mm with a standard deviation of 1.3219 and

Contracted Right Masseter length obtained was 12.49 mm with a standard deviation of 1.5317. The p value obtained was 0.000 which is < 0.001 and is highly significant.

Table 3: Relaxed Left Masseter and Contracted Left Masseter measurements in Control group

This table denotes Relaxed and Contracted muscle lengths of Left side Masseter muscle of Control group. The mean Relaxed Left Masseter length obtained was 8.940 mm with a standard deviation of 1.5209 and Contracted Left masseter measurements obtained were 12.410 mm with a standard deviation of 1.5082. The p value obtained was 0.000 which is < 0.001 and is highly significant.

Table 4: Relaxed Right Masseter and Relaxed left Masseter measurements in Control group

This table denotes Relaxed Right and Left side Masseter muscle lengths in Control group. The mean relaxed right Masseter length obtained was 8.70 mm with a standard deviation of 1.3219 and relaxed left Masseter length obtained was 8.94 mm with a standard deviation of 1.5209. The p value obtained was 0.417 which is > 0.05 and is insignificant.

Table 5: Contracted Right Masseter and Contracted left Masseter measurements in Control group

This table denotes Contracted Right and left side Masseter muscle lengths in Control group. The mean Contracted Right Masseter length obtained was 12.490 mm with a standard deviation of 1.5317. The mean Contracted Left Masseter length obtained was 12.410 mm with a standard deviation of 1.5082. The p value obtained was 0.778 which is > 0.05 and is insignificant.

Table 6: Relaxed Right Masseter and Contracted Right Masseter measurements in Study group

This table denotes Relaxed and Contracted muscle lengths of Right side Masseter muscle of Study group. The mean Relaxed Right Masseter length obtained was 10.360 mm with a standard deviation of 1.6298 and Contracted Right Masseter measurements obtained were 14.865 mm with a standard deviation of 1.8658. The p value obtained was 0.000 which is < 0.001 and is highly significant

Table 7: Relaxed Left Masseter and Contracted Left Masseter measurements in Study group

This table denotes Relaxed and Contracted muscle lengths of Left side Masseter muscle of Study group. The mean Relaxed Left Masseter length obtained was 10.785 mm with a standard deviation of 1.5315 and Contracted

Left masseter measurements obtained were 15.535 mm with a standard deviation of 2.4508. The p value obtained was 0.000 which is < 0.001 and is highly significant.

Table 8: Relaxed Right Masseter and Relaxed left Masseter measurements in Study group

This table denotes Relaxed Right and Left side Masseter muscle lengths in Study group. The mean relaxed right Masseter length obtained was 10.36 mm with a standard deviation of 1.6298 and relaxed left Masseter length obtained was 10.785 mm with a standard deviation of 1.5315. The p value obtained was 0.297 which is > 0.05 and is insignificant.

Table 9: Contracted Right Masseter and Contracted left Masseter measurements in Study group

This table denotes Contracted Right and left side Masseter muscle lengths in Study group. The mean Contracted Right Masseter length obtained was 14.865 mm with a standard deviation of 1.8658. The mean Contracted Left Masseter length obtained was 15.535 mm with a standard deviation of 2.4508. The p value obtained was 0.207 which is > 0.05 and is insignificant.

Table 10: Relaxed Right Masseter measurements in Control and Study groups

This table denotes the Comparison of Relaxed Right side Masseter muscle lengths in Control and Study group. The mean relaxed right masseter length obtained in the Control group was 8.70 mm with a standard deviation of 1.3219 and in the Study group was 10.36 mm with a standard deviation of 1.6298. The p value obtained was 0.001 which is < 0.05 and is Significant.

Table 11: Relaxed Left Masseter measurements in Control and Study groups

This table denotes the Comparison of Relaxed Left side Masseter muscle lengths in Control and Study group. The mean relaxed left masseter length in the Control group was 8.940 mm with a standard deviation of 1.5209 and in the Study group was 10.785 mm with a standard deviation of 1.5315. The p value obtained was 0.000 which is < 0.001 and is highly significant.

Table 12: Contracted Right Masseter measurements in Control and Study groups

This table denotes the Comparison of Contracted Right side Masseter muscle lengths in Control and Study group. The mean contracted right masseter length obtained in the Control group was 12.490 mm with a standard deviation of 1.5317 and in the Study group was 14.865 mm with a standard

deviation of 1.8658. The p value obtained was 0.000 which is < 0.001 and is highly significant.

Table 13: Contracted Left Masseter measurements in Control and Study groups

This table denotes the Comparison of Contracted Left side Masseter muscle lengths in Control and Study group. The mean contracted left masseter length obtained in the Control group was 12.41 mm with a standard deviation of 1.5082 and in the Study group was 15.535 mm with a standard deviation of 2.4508. The p value obtained was 0.000 which is < 0.001 and is highly significant.

Table 14: Relaxed Right Masseter and Contracted Right Masseter measurements in Control and Study groups

This table denotes the Comparison of Relaxed and Contracted Right side Masseter muscle lengths in Control and Study group. The mean relaxed right masseter length obtained in the Control group was 8.70 mm with a standard deviation of 1.3219 and in the Study group was 10.36 mm with a standard deviation of 1.6298. The mean Contracted right masseter length obtained in the Control group was 12.49 with a standard deviation of 1.5317 and in Study group was 14.865 with a standard deviation of 1.8658. The p value obtained was 0.000 which is < 0.001 and is highly significant.

Table 15: Relaxed Left Masseter and Contracted Left Masseter measurements in Control and Study groups

This table denotes the Comparison of Relaxed and Contracted Left side Masseter muscle lengths in Control and Study group. The mean relaxed left masseter length obtained in the Control group was 8.94 mm with a standard deviation of 1.5209 and in the Study group was 10.785 mm with a standard deviation of 1.5315. The mean Contracted left masseter length obtained in the Control group was 12.41 mm with a standard deviation of 1.5082 and in Study group was 15.535 mm with a standard deviation of 2.4508. The p value obtained was 0.000 which is < 0.001 and is highly significant.

Table 16: Side wise distribution of subjects in Control group

This Table shows the distribution of subjects based on the habitual chewing side in the Control group. Out of the 20 subjects in Control group 13 (65%) of the subjects frequently use Right side for chewing and 7 (35%) of the subjects use Left side for chewing .The p value obtained was 0.337 which is > 0.05 and is Insignificant.

Table 17: Side wise distribution of subjects in Study group

This Table shows the distribution of subjects based on the side frequently used for Tobacco chewing in the Study group. Out of the 20 subjects in Study group 10 (50.0%) of the subjects frequently use Right side for chewing and 10 (50.0%) of the subjects use Left side for chewing .The p value obtained was 1.000 which is > 0.05 and is insignificant.

Table 18: Side wise distribution of subjects in Control and Study groups

This Table shows the distribution of subjects based on the Right side and Left side usage for habitual chewing in Control group and Tobacco chewing in the Study group. Out of the 40 subjects in Control and Study group 23(57.5%) of them among which 13(65%) of the controls and 10 (50%) of the study group subjects are Right side chewers and 17(42.5%) of them out of which 7 (35%) of the Controls and 10 (50%) of the subjects who belong to Study group are Left side Chewers. The p value obtained was 0.337 which is > 0.05 and is insignificant.

Table 19: Detection of branch of transverse facial artery in Control group

This table shows distribution of subjects with branch of transverse facial artery detected in control group where out of 20 controls, in 7 (35%) of them artery could be detected whereas in 13 (65%) of them it is not detected. The p value obtained was 0.337 which is > 0.05 and is insignificant.

Table 20: Detection of branch of transverse facial artery in Study group

This table shows distribution of subjects with branch of transverse facial artery detected in study group where out of 20 subjects in Study group, in 10 (50%) of them artery could be detected whereas in 10 (50%) of them it could not be detected. The p value obtained was 1.000 which is > 0.05 and is insignificant.

Table 21: Detection of branch of transverse facial artery in control and study groups

This Table shows the detection of branch of transverse facial artery in control and study groups. Out of the 40 subjects in Control and Study groups in 17(42.5%) of them among which 7(35%) of the control and 10 (50%) of the study group subjects artery was detected and in 23(57.5%) of them out of which 13 (65%) of the Controls and 10 (50%) of the subjects who belong to Study group artery was not detected .The p value obtained was 0.337 which is > 0.05 and is insignificant.

Table 22: Correlation between relaxed right masseter measurement and side used in study group

This table shows correlation between Relaxed Right masseter length and side used. Among the 10 right and 10 left side users the mean relaxed right masseter values obtained were 10.46 mm with a standard deviation of 1.84 and 10.26 mm with a standard deviation of 1.48 respectively. The

pearson correlation was utilized. The p value obtained was 0.792 which is > 0.05 and is insignificant.

Table 23: Correlation between contracted right masseter measurement and side used in study group

This table shows correlation between Contracted Right masseter length and side used. Among the 10 right and 10 left side users the mean contracted right masseter values obtained were 14.82 mm with a standard deviation of 1.97 and 14.91 mm with a standard deviation of 1.85 respectively. The pearson correlation was utilized. The p value obtained was 0.918 which is > 0.05 and is insignificant.

Table 24: Correlation between relaxed left masseter measurement and side used in study group

This table shows correlation between relaxed left masseter length and side used. Among the 10 right and 10 left side users the mean relaxed left masseter values obtained were 10.06 mm with a standard deviation of 1.18 and 11.51mm with a standard deviation of 1.54 respectively. The pearson correlation was utilized. The p value obtained was 0.030 which is < 0.05 and is significant.

Table 25: Correlation between contracted left masseter measurement and side used in study group

This table shows correlation between Contracted left masseter length and side used. Among the 10 right and 10 left side users the mean contracted left masseter values obtained were 13.83 mm with a standard deviation of 1.51 and 17.24 mm with a standard deviation of 1.98 respectively. The pearson correlation was utilized. The p value obtained was 0.000 which is < 0.001 and is highly significant.

Table 26: Correlation between Relaxed right masseter measurements and number of packets of tobacco consumed per day in Study group

This table shows correlation between relaxed right masseter length and number of packets of tobacco consumed per day in Study group. The number of packets taken per day were divided into 3-7 and 8-15. There were an equal number of 10 subjects in each with a mean relaxed Right masseter values of 10.12 mm with a standard deviation of 0.99 and 10.60 mm with a standard deviation of 2.11 in 3-7 and 8-15 packets per day respectively. The Pearson Correlation was utilized. The p value obtained was 0.525 which is > 0.05 and is insignificant.

Table 27: Correlation between Contracted right masseter measurement and number of packets of tobacco consumed per day in Study group

This table shows correlation between Contracted right masseter length and number of packets of tobacco consumed per day in Study group. The number of packets taken per day were divided into 3-7 and 8-15. There were an equal number of 10 subjects in each with a mean contracted Right masseter values of 14.77 mm with a standard deviation of 1.24 and 14.96 mm with a standard deviation 2.40 in 3-7 and 8-15 packets per day respectively. The Pearson Correlation was utilized. The Pearson Correlation was utilized. The p value obtained was 0.827 which is > 0.05 and is insignificant.

Table 28: Correlation between Relaxed left masseter measurement and number of packets of tobacco consumed per day in Study group

This table shows correlation between relaxed left masseter length and number of packets of tobacco consumed per day in Study group. The number of packets taken per day were divided into 3-7 and 8-15. There were an equal number of 10 subjects in each with a mean contracted Right masseter values of 10.88 mm with a standard deviation of 1.57 and 10.69 mm with a standard deviation of 1.56 in 3-7 and 8-15 packets per day respectively. The Pearson Correlation was utilized. The p value obtained was 0.790 which is > 0.05 and is insignificant.

Table 29: Correlation between Contracted left masseter measurement and number of packets of tobacco consumed per day in Study group

This table shows correlation between Contracted left masseter length and number of packets of tobacco consumed per day in Study group. The number of packets taken per day were divided into 3-7 and 8-15. There were an equal number of 10 subjects in each with a mean contracted Right masseter values of 16.04 mm with a standard deviation of 2.43 and 15.03 mm with a standard deviation 2.48 in 3-7 and 8-15 packets per day respectively. The Pearson Correlation was utilized. The Pearson Correlation was utilized. The p value obtained was 0.371 which is > 0.05 and is insignificant.

Table 30: Correlation between Relaxed right masseter measurements and number of years of chewing in Study group

This table shows correlation between relaxed right masseter length and number of years of chewing in Study group. The number of years of chewing were divided into 3-7 and 8-15. There were 13 subjects with 3-7 years of chewing and 7 subjects with 8-15 years with a mean relaxed Right masseter values of 10.17 mm with a standard deviation of 1.66 and 10.70 mm with a standard deviation 1.63 respectively. The Pearson Correlation was utilized. The p value obtained was 0.508 which is > 0.05 and is insignificant.

Table 31: Correlation between Contracted right masseter measurement and number of years of chewing in Study group

This table shows correlation between Contracted right masseter length and number of years of chewing in Study group. The number of years of chewing were divided into 3-7 and 8-15. There were 13 subjects with 3-7 years of chewing and 7 subjects with 8-15 years with a mean contracted Right masseter values of 14.89 mm with a standard deviation of 1.74 and 14.81 mm with a standard deviation 2.22 respectively. The Pearson Correlation was utilized. The Pearson Correlation was utilized. The p value obtained was 0.932 which is > 0.05 and is insignificant.

Table 32: Correlation between Relaxed left masseter measurement and number of years of chewing in Study group

This table shows correlation between relaxed left masseter length and number of years of chewing in Study group. The number of years of chewing were divided into 3-7 and 8-15. There were 13 subjects with 3-7 years of chewing and 7 subjects with 8-15 years with a mean relaxed Left masseter values of 10.38 mm with a standard deviation of 1.14 and 11.52 mm with a standard deviation 1.95 respectively. The Pearson Correlation was utilized. The p value obtained was 0.113 which is >0.05 and is insignificant.

Table 33: Correlation between Contracted left masseter measurement and number of years of chewing in Study group

This table shows correlation between Contracted left masseter length and number of years of chewing in Study group. The number of years of chewing were divided into 3-7 and 8-15. There were 13 subjects with 3-7 years of chewing and 7 subjects with 8-15 years with a mean contracted left masseter values of 15.36 mm with a standard deviation of 2.30 and 15.85 mm with a standard deviation 2.86 respectively The Pearson Correlation was utilized. The p value obtained was 0.678 which is > 0.05 and is insignificant.

Table 34: Correlation between Relaxed right masseter measurement and age in Study group

This table shows correlation between relaxed right masseter length and age in Study group. The mean relaxed right masseter values obtained for the age group of 21-30 and 31-40 years was 9.88 mm with a standard deviation of 1.04 and 10.84 mm with a standard deviation of 1.99 respectively. The Pearson Correlation was utilized. The p value obtained was 0.195 which is > 0.05 and is insignificant.

Table 35: Correlation between Contracted right masseter measurement and age in Study group

This table shows correlation between Contracted right masseter length and age in Study group. The mean contracted right masseter values obtained

for the age group of 21-30 and 31-40 years was 13.70 mm with a standard deviation of 1.21 and 16.03 mm with a standard deviation of 1.69 respectively. The Pearson Correlation was utilized. The p value obtained was 0.002 which is < 0.05 and is Significant.

Table 36: Correlation between Relaxed left masseter measurement and age in Study group

This table shows correlation between relaxed left masseter length and age in study group. The mean relaxed left masseter values obtained for the age group of 21-30 and 31-40 years was 10.14 mm with a standard deviation of 1.46 and 11.43 mm with a standard deviation of 1.37 respectively. The Pearson Correlation was utilized. The p value obtained was 0.057 which is > 0.05 and is insignificant.

Table 37: Correlation between Contracted left masseter measurement and age in Study group

This table shows correlation between contracted left masseter length and age in study group. The mean relaxed right masseter values obtained for the age group of 21-30 and 31-40 years was 14.68 mm with a standard deviation of 2.33 and 16.39 mm with a standard deviation of 2.37 respectively. The Pearson Correlation was utilized. The p value obtained was 0.121 which is > 0.05 and is insignificant.

Table 38: Correlation between right side chewing and artery detection in Study group

This table shows Correlation between Right side chewing and artery detection in Study group. Among the 10 subjects of study group who are right side chewers artery was detected on right side in 2 (20%) and not detected in 8 (80%) subjects. The Pearson Correlation was utilized. The p value obtained was 0.068 which is > 0.05 and is insignificant.

Table 39: Correlation between Left side chewing and artery detection in Study group

This table shows correlation between Left side chewing and artery detection in Study group. Among the 10 subjects of study group who are left side chewers artery was detected on left side in 4 (40%) and not detected in 6 (60%) subjects. The Pearson Correlation was utilized. The p value obtained was 1.000 which is > 0.05 and is insignificant.

Table 1: Age comparison of subjects in control and study groups

Variable	Group	N	Mean	Std. Deviation	P Value
Age in years	Control	20	28.55	6.160	0.074
	Study	20	31.90	5.350	

Table 2: Relaxed right masseter and contracted right masseter measurements in control group

Muscle parameters	Group	N	Mean	Std. Deviation	P value
Relaxed Right Masseter	Control	20	8.700	1.3219	0.000
Contracted Right Masseter	Control	20	12.490	1.5317	

Table 3: Relaxed left masseter and contracted left masseter measurements in control group

Muscle parameters	Group	N	Mean	Std. Deviation	P value
Relaxed left Masseter	Control	20	8.940	1.5209	0.000
Contracted Left Masseter	Control	20	12.410	1.5082	

Table 4: Relaxed right masseter and relaxed left masseter measurements in control group

Muscle parameters	Group	N	Mean	Std.Deviation	P value
Relaxed Right Masseter	Control	20	8.700	1.3219	0.417
Relaxed Left Masseter	Control	20	8.940	1.5209	

Table 5: Contracted right masseter and contracted left masseter measurements in control group

Muscle parameters	Group	N	Mean	Std. Deviation	P value
Contracted Right Masseter	Control	20	12.490	1.5317	0.778
Contracted Left Masseter	Control	20	12.410	1.5082	

Table 6: Relaxed right masseter and contracted right masseter measurements in study group

Muscle parameters	Group	N	Mean	Std. Deviation	P value
Relaxed Right Masseter	Study	20	10.360	1.6298	0.000
Contracted Right Masseter	Study	20	14.865	1.8658	

Table 7: Relaxed left masseter and contracted left masseter measurements in study group

Muscle parameters	Group	N	Mean	Std. Deviation	P value
Relaxed Left Masseter	Study	20	10.785	1.5315	0.000
Contracted left Masseter	Study	20	15.535	2.4508	

Table 8: Relaxed right masseter and relaxed left masseter measurements in study group

Muscle parameters	Group	N	Mean	Std. Deviation	P value
Relaxed Right Masseter	Study	20	10.360	1.6298	0.297
Relaxed left Masseter	Study	20	10.785	1.5315	

Table 9: Contracted right masseter and contracted left masseter measurements in study group

Muscle parameters	Group	N	Mean	Std. Deviation	P value
Contracted Right Masseter	Study	20	14.865	1.8658	0.207
Contracted Left Masseter	Study	20	15.535	2.4508	

Table 10: Relaxed right masseter measurements in control and study groups

Muscle parameter	Group	N	Mean	Std. Deviation	P value
Relaxed Right Masseter	Control	20	8.700	1.3219	0.001
	Study	20	10.360	1.6298	

Table 11: Relaxed left masseter measurements in control and study groups

Muscle parameter	Group	N	Mean	Std. Deviation	P value
Relaxed Left Masseter	Control	20	8.940	1.5209	0.000
	Study	20	10.785	1.5315	

Table 12: Contracted right masseter measurements in control and study groups

Muscle parameter	Group	N	Mean	Std. Deviation	P value
Contracted Right Masseter	Control	20	12.490	1.5317	0.000
	Study	20	14.865	1.8658	

Table 13: Contracted left masseter measurements in control and study groups

Muscle parameter	Group	N	Mean	Std. Deviation	P value
Contracted Left Masseter	Control	20	12.41	1.5082	0.000
	Study	20	15.535	2.4508	

Table 14: Relaxed right masseter and contracted right masseter measurements in control and study groups

Muscle parameters	Group	N	Mean	Std. Deviation	P value
Relaxed Right Masseter	Control	20	8.70	1.3219	0.000
	Study	20	10.36	1.6298	
Contracted Right Masseter	Control	20	12.49	1.5317	
	Study	20	14.865	1.8658	

Table 15: Relaxed left masseter and contracted left masseter measurements in control and study groups

Muscle parameters	Group	N	Mean	Std. Deviation	P value
Relaxed Left Masseter	Control	20	8.940	1.5209	0.000
	Study	20	10.785	1.5315	
Contracted Left Masseter	Control	20	12.410	1.5082	
	Study	20	15.535	2.4508	

Table 16: Side wise distribution of subjects in control group

Side Used	Control Group	P value
Right	13 (65%)	0.337
Left	7 (35%)	
Total	20 (100%)	

Table 17: Side wise distribution of subjects in study group

Side Used	Study Group	P value
Right	10 (50%)	1.000
Left	10 (50%)	
Total	20 (100%)	

Table 18: Side wise distribution of subjects in control and study groups

Side used	Group		Total	P value
	Control	Study		0.337
Right	13(65%)	10(50%)	23(57.5%)	
Left	7 (35%)	10(50%)	17(42.5%)	
Total	20(100%)	20(100%)	40(100%)	

Table 19: Detection of branch of transverse facial artery in control group

Artery detection	Group	P value
	Control	
Yes	7 (35%)	0.337
No	13 (65%)	
Total	20 (100%)	

Table 20: Detection of branch of transverse facial artery in study group

Artery detection	Group	P value
	Study	
Yes	10 (50%)	1.000
No	10 (50%)	
Total	20 (100%)	

Table 21: Detection of branch of transverse facial artery in control and study groups

Artery detection	Group		Total	P value
	Control	Study		
Yes	7 (35%)	10(50%)	17(42.5%)	0.337
No	13 (65%)	10(50%)	23(57.5%)	
Total	20(100%)	20(100%)	40(100%)	

Table 22: Correlation between relaxed right masseter measurement and side used in study group

Muscle parameter	Side used	N	Mean	Std. Deviation	P value
Relaxed Right masseter	Right	10	10.46	1.84	0.792
	Left	10	10.26	1.48	

Table 23: Correlation between contracted right masseter measurement and side used in study group

Muscle parameter	Side used	N	Mean	Std. Deviation	P value
Contracted Right masseter	Right	10	14.82	1.97	0.918
	Left	10	14.91	1.85	

Table 24: Correlation between relaxed left masseter measurement and side used in study group

Muscle parameter	Side used	N	Mean	Std. Deviation	P value
Relaxed Left masseter	Right	10	10.06	1.18	0.030
	Left	10	11.51	1.54	

Table 25: Correlation between contracted left masseter measurement and side used in study group

Muscle parameter	Side used	N	Mean	Std. Deviation	P value
Contracted Left masseter	Right	10	13.83	1.51	0.000
	Left	10	17.24	1.98	

Table 26: Correlation between relaxed right masseter measurement and number of packets of tobacco consumed per day in study group

Muscle parameter	No. of packets per day	N	Mean	Std. Deviation	P value
Relaxed Right masseter	3-7	10	10.12	0.99	0.525
	8-15	10	10.60	2.11	

Table 27: Correlation between contracted right masseter measurement and number of packets of tobacco consumed per day in study group

Muscle parameter	No. of packets per day	N	Mean	Std. Deviation	P value
Contracted Right masseter	3-7	10	14.77	1.24	0.827
	8-15	10	14.96	2.40	

Table 28: Correlation between relaxed left masseter measurement and number of packets of tobacco consumed per day in study group

Muscle parameter	No. of packets per day	N	Mean	Std. Deviation	P value
Relaxed left masseter	3-7	10	10.88	1.57	0.790
	8-15	10	10.69	1.56	

Table 29: Correlation between contracted left masseter measurement and number of packets of tobacco consumed per day in study group

Muscle parameter	No. of packets per day	N	Mean	Std. Deviation	P value
Contracted left masseter	3-7	10	16.04	2.43	0.371
	8-15	10	15.03	2.48	

Table 30: Correlation between relaxed right masseter measurement and number of years of chewing in study group

Muscle parameter	No. of years of chewing	N	Mean	Std. Deviation	P value
Relaxed Right masseter	3-7	13	10.17	1.66	0.508
	8-15	7	10.70	1.63	

Table 31: Correlation between contracted right masseter measurement and number of years of chewing in study group

Muscle parameter	No. of years of chewing	N	Mean	Std. Deviation	P value
Contracted Right masseter	3-7	13	14.89	1.74	0.932
	8-15	7	14.81	2.22	

Table 32: Correlation between relaxed left masseter measurement and number of years of chewing in study group

Muscle parameter	No. of years of chewing	N	Mean	Std. Deviation	P value
Relaxed Left masseter	3-7	13	10.38	1.14	0.113
	8-15	7	11.52	1.95	

Table 33: Correlation between contracted left masseter measurement and number of years of chewing in study group

Muscle parameter	No. of years of chewing	N	Mean	Std. Deviation	P value
Contracted left masseter	3-7	13	15.36	2.30	0.678
	8-15	7	15.85	2.86	

Table 34: Correlation between relaxed right masseter measurement and age in study group

Muscle parameter	Age Group in years	N	Mean	Std. Deviation	P value
Relaxed Right masseter	21-30	10	9.88	1.0486	0.195
	31-40	10	10.84	1.9990	

Table 35: Correlation between contracted right masseter measurement and age in study group

Muscle parameter	Age Group in years	N	Mean	Std. Deviation	P value
Contracted Right masseter	21-30	10	13.70	1.2147	0.002
	31-40	10	16.03	1.6905	

Table 36: Correlation between relaxed left masseter measurement and age in study group

Muscle parameter	Age Group in years	N	Mean	Std. Deviation	P value
Relaxed Left masseter	21-30	10	10.140	1.4615	0.057
	31-40	10	11.430	1.3752	

Table 37: Correlation between contracted left masseter measurement and age in study group

Muscle parameter	Age Group in years	N	Mean	Std. Deviation	P value
Contracted Left masseter	21-30	10	14.68	2.3309	0.121
	31-40	10	16.39	2.3713	

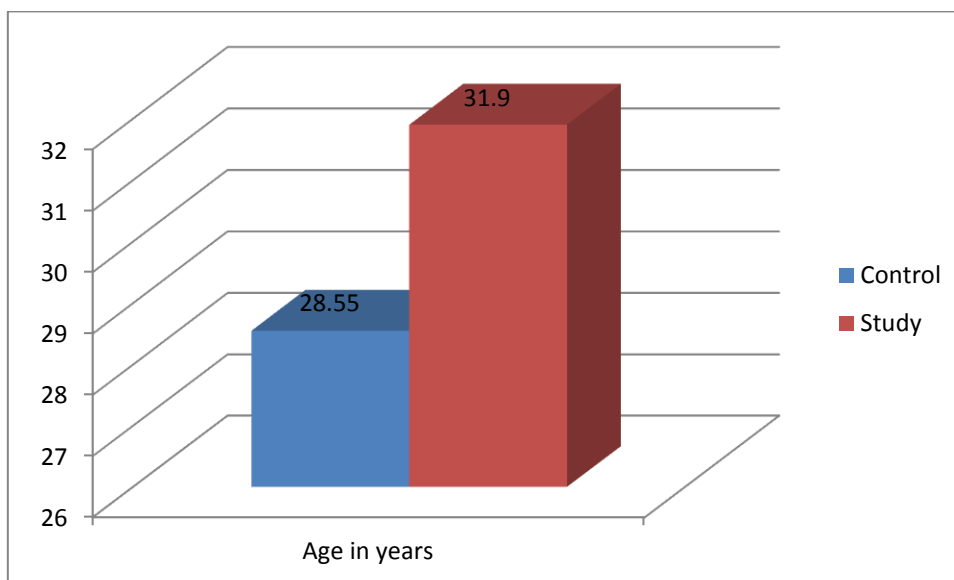
Table 38: Correlation between right side chewing and artery detection in study group

Side used	Artery detection			P value
	Yes	No	Total	0.068
Right	2 (20%)	8 (80%)	10 (100%)	

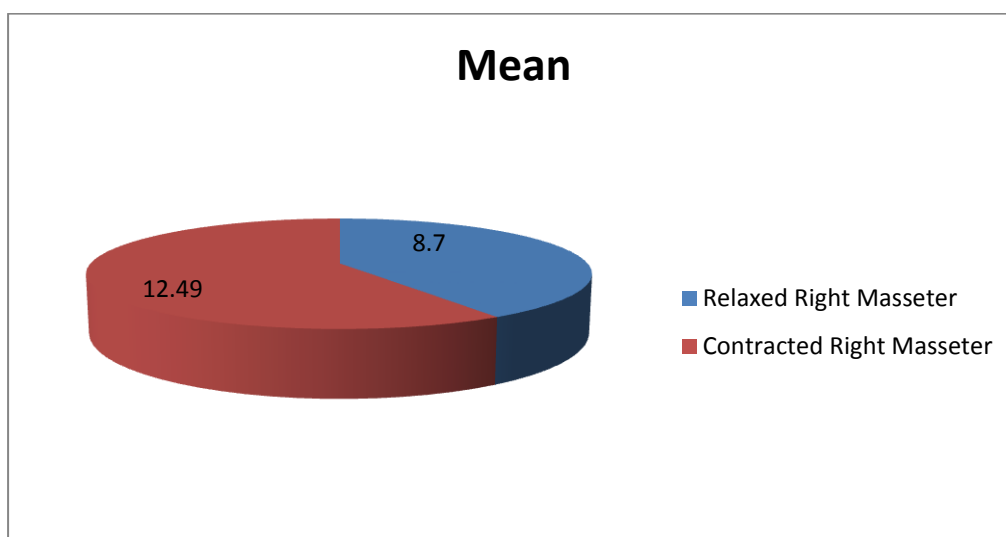
Table 39: Correlation between left side chewing and artery detection in study group

Side used	Artery detection			P value
	Yes	No	Total	1.000
Left	4 (40%)	6 (60%)	10 (100%)	

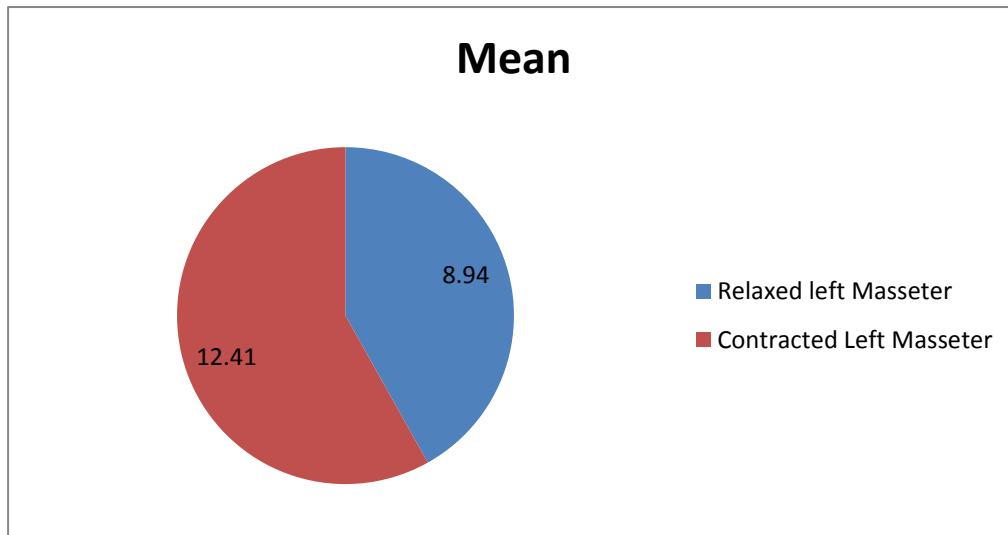
Graph-1: Age comparison of subjects in control and study groups



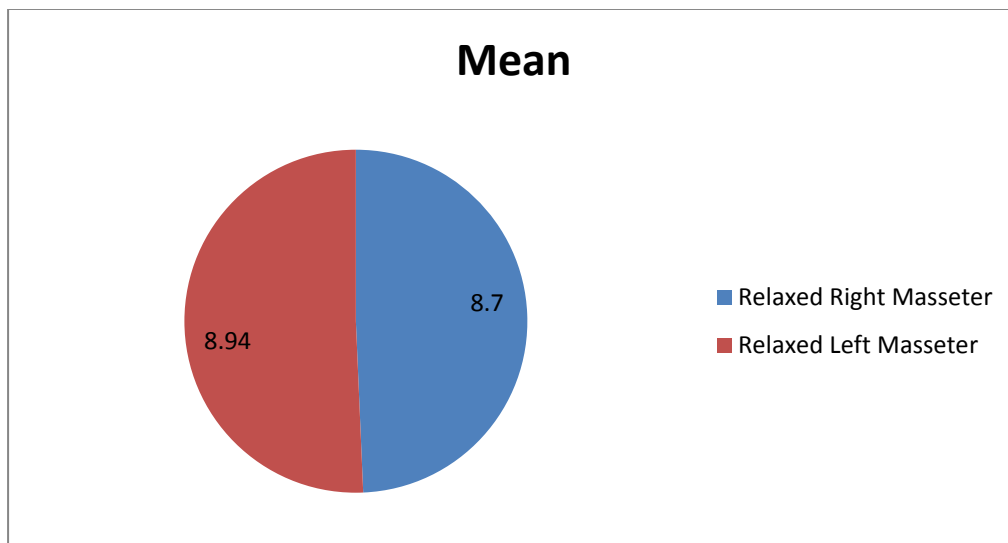
Graph-2: Relaxed right masseter and contracted right masseter measurements in control group



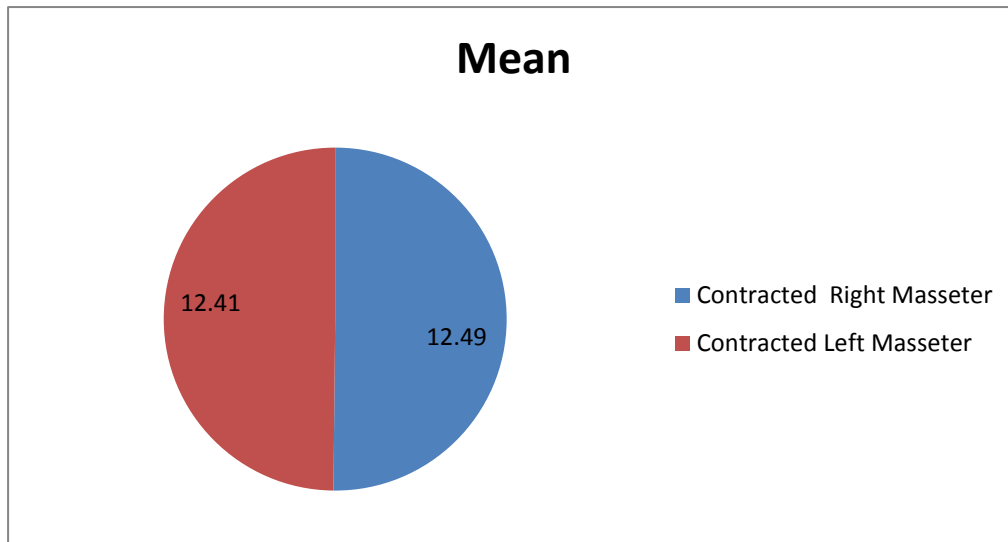
**Graph-3: Relaxed left masseter and contracted left masseter
measurements in control group**



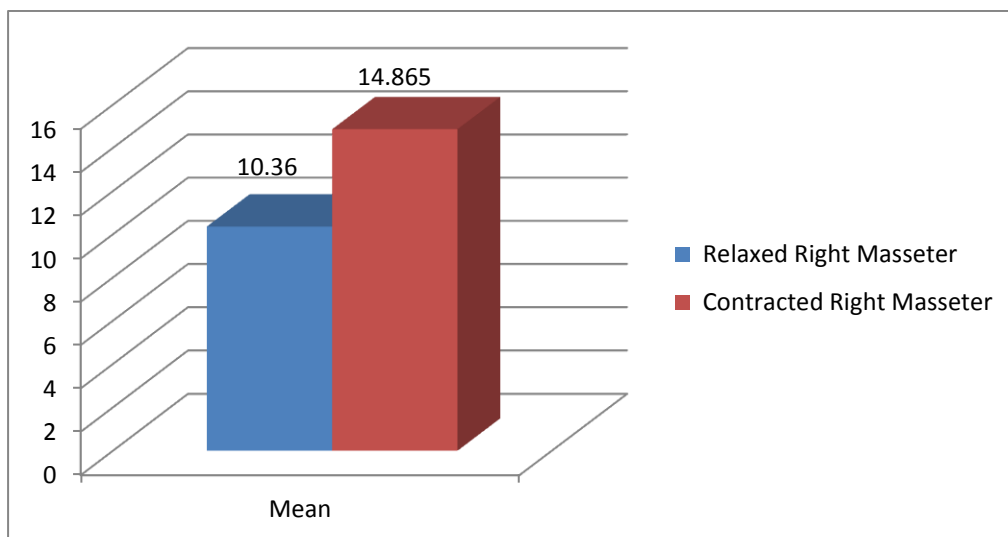
**Graph-4: Relaxed right masseter and relaxed left masseter measurements
in control group**



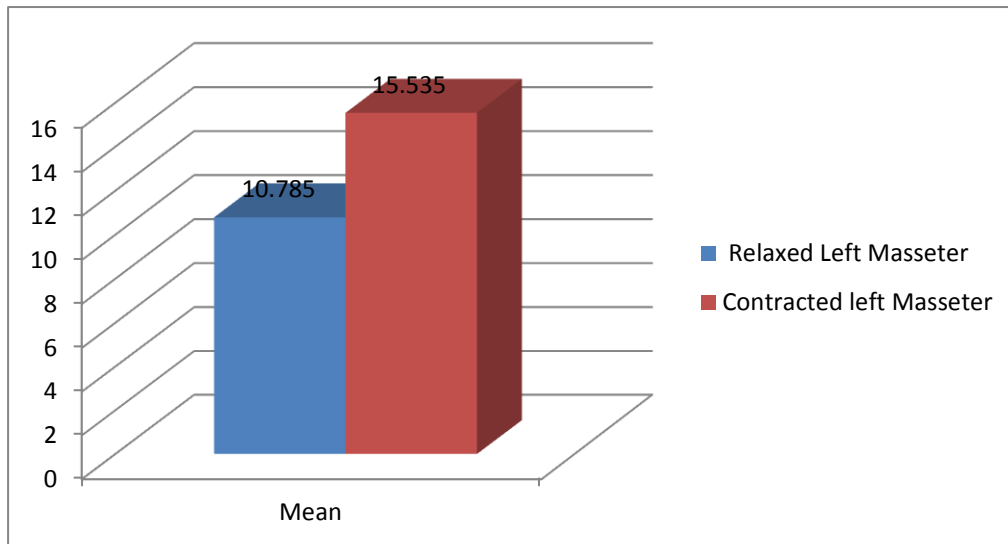
**Graph-5: Contracted right masseter and contracted left masseter
measurements in control group**



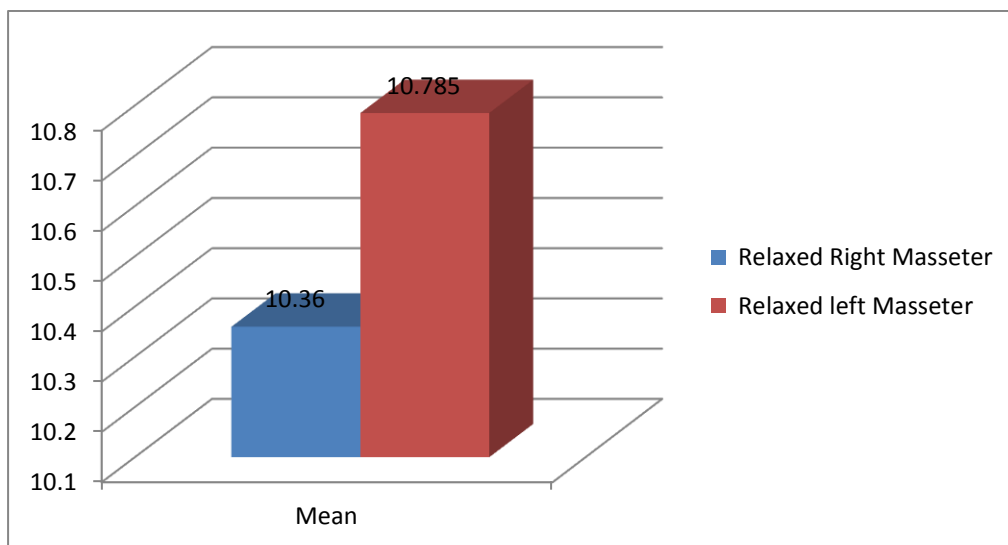
**Graph-6: Relaxed right masseter and contracted right masseter
measurements in study group**



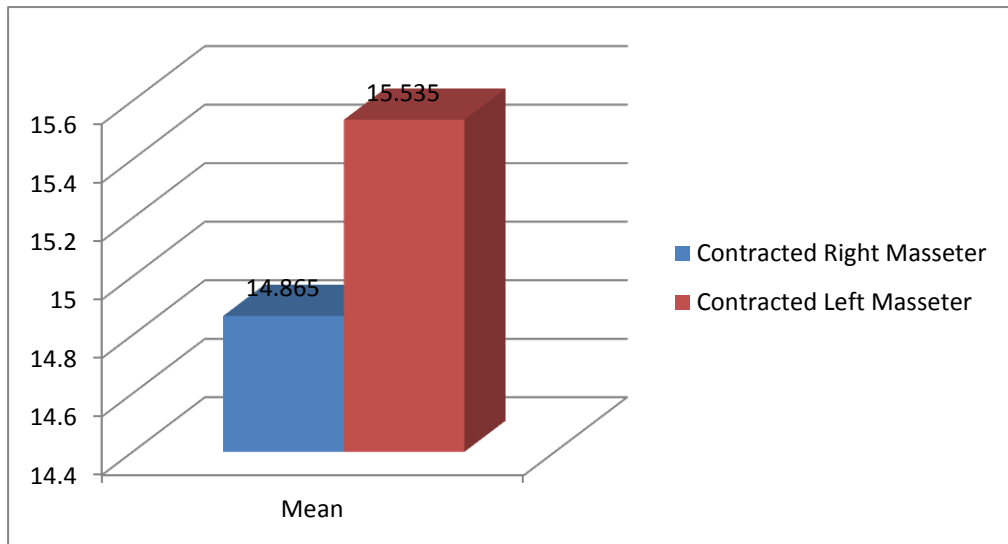
Graph-7: Relaxed left masseter and contracted left masseter measurements in study group



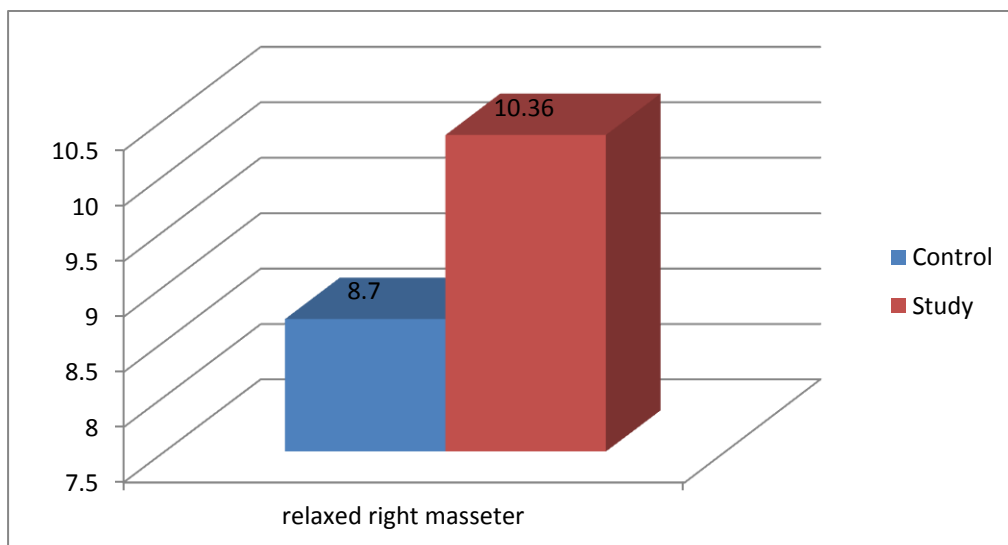
Graph-8: Relaxed right masseter and relaxed left masseter measurements in study group



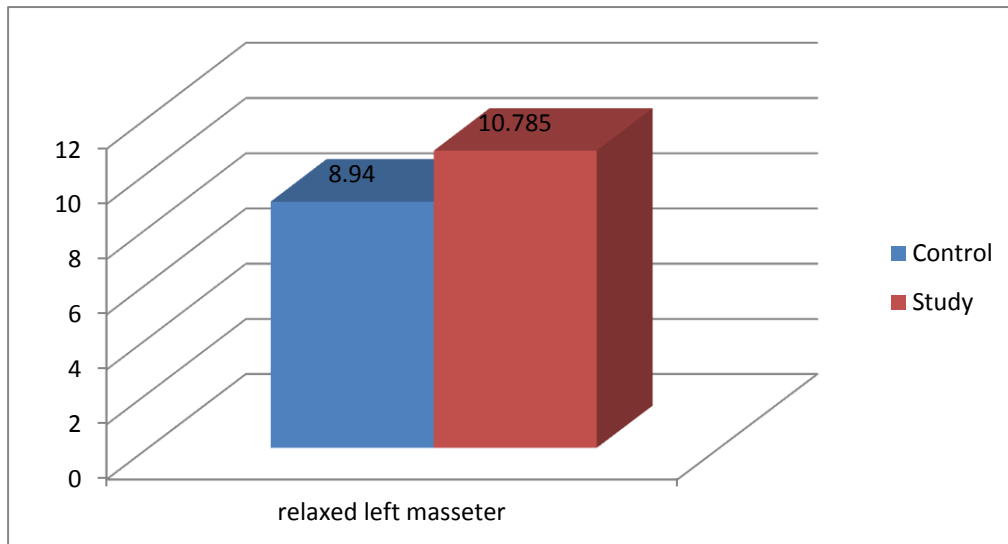
Graph-9: Contracted right masseter and contracted left masseter measurements in study group



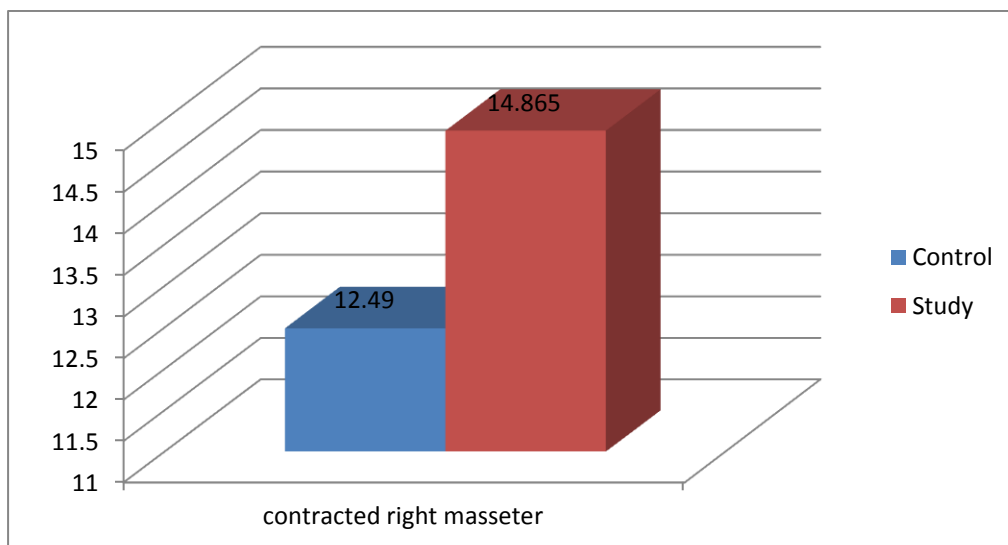
Graph-10: Relaxed right masseter measurements in control and study groups



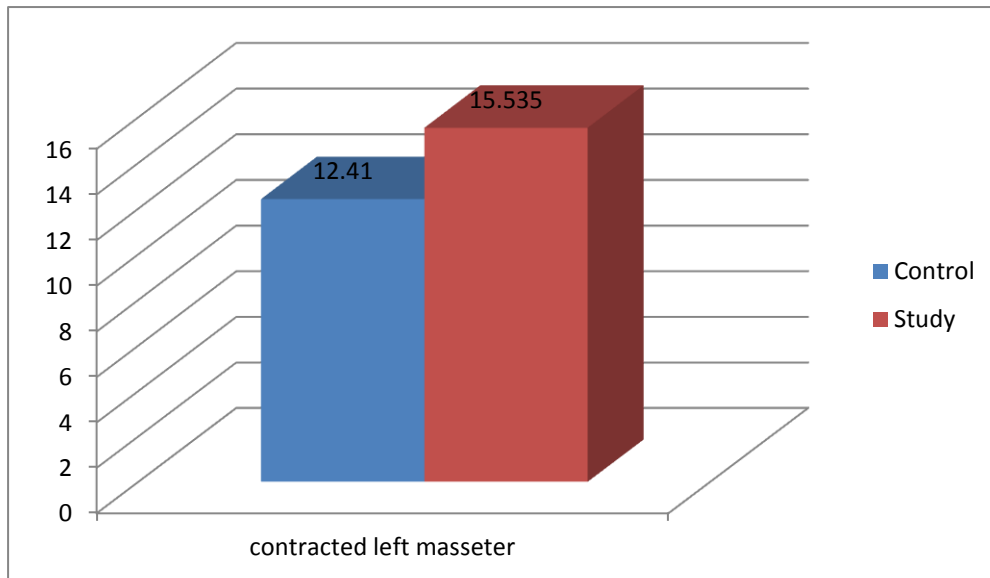
Graph-11: Relaxed left masseter measurements in control and study groups



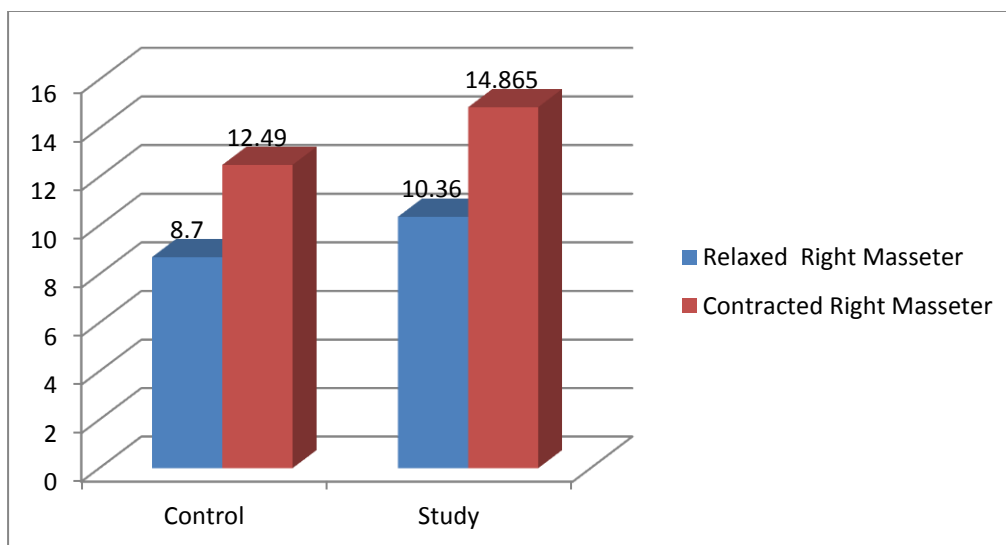
Graph-12: Contracted right masseter measurements in control and study groups



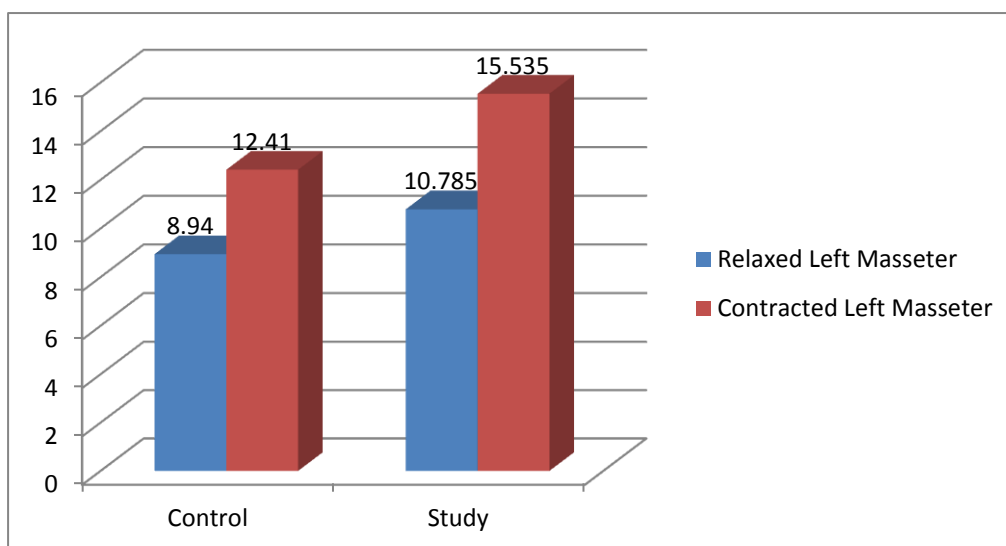
Graph-13: Contracted left masseter measurements in control and study groups



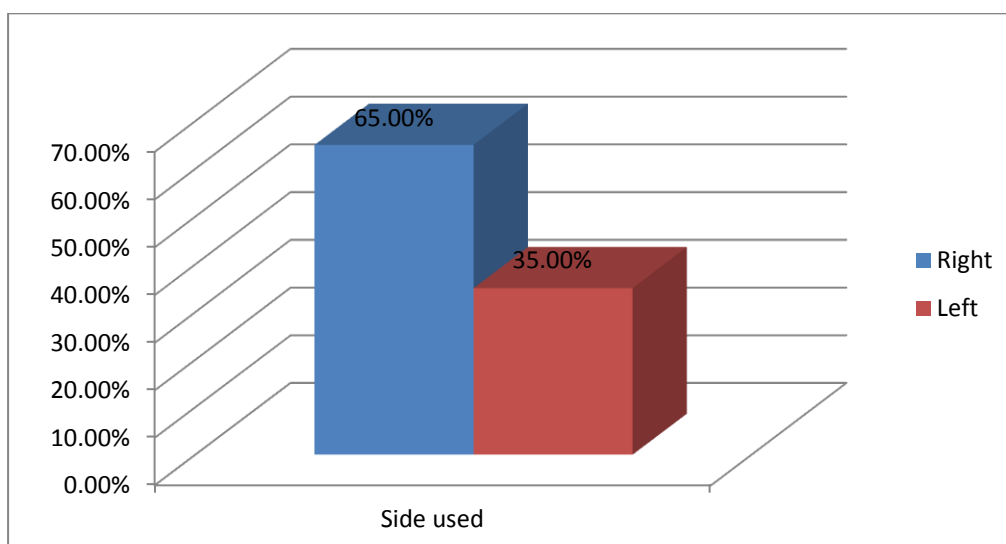
Graph-14: Relaxed right masseter and contracted right masseter measurements in control and study groups



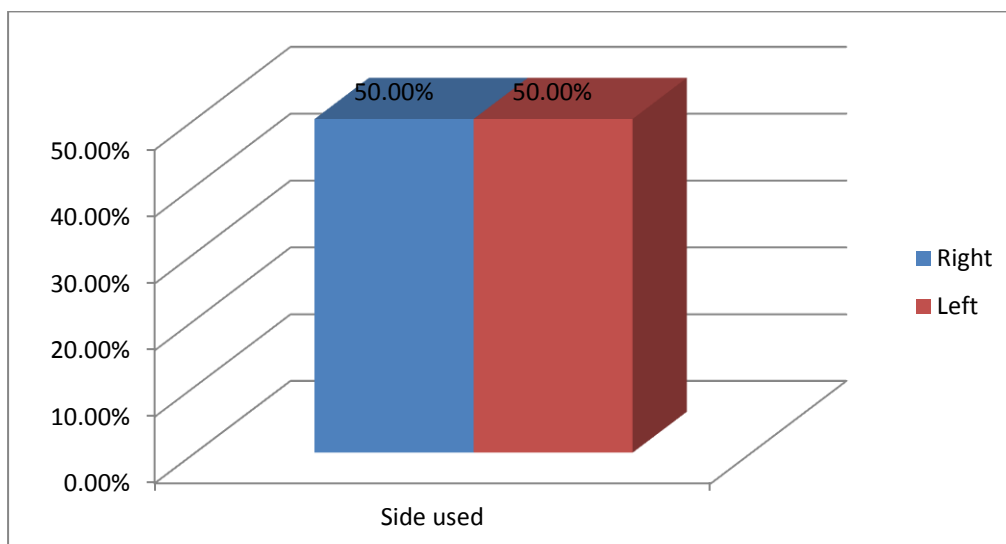
Graph-15: Relaxed left masseter and contracted left masseter measurements in control and study groups



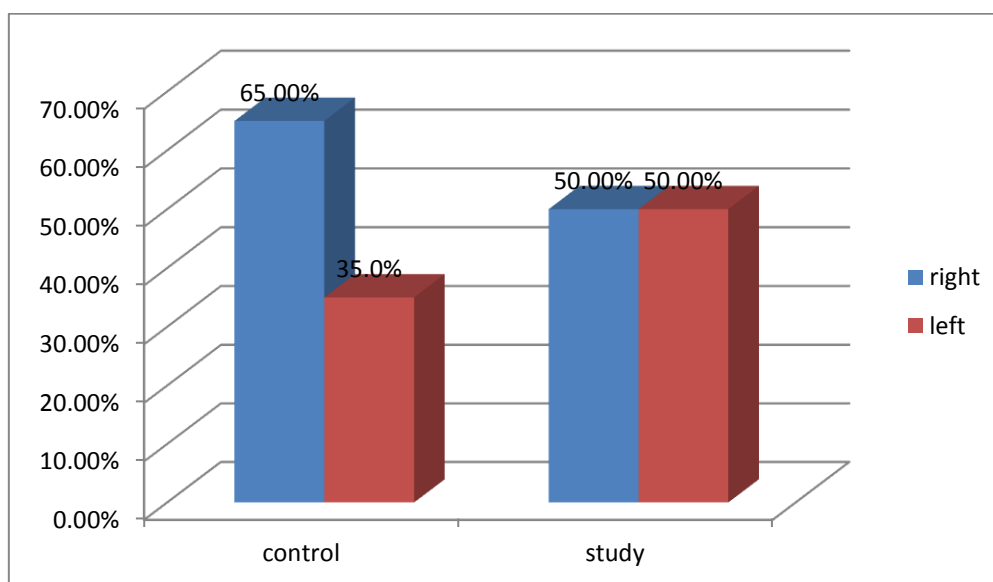
Graph-16: Side wise distribution of subjects in control group



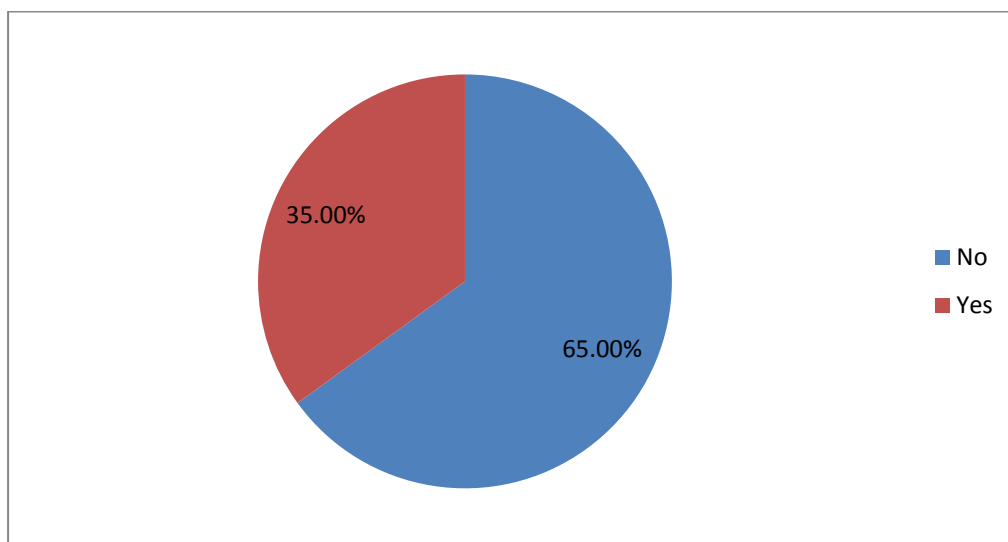
Graph-17: Side wise distribution of subjects in study group



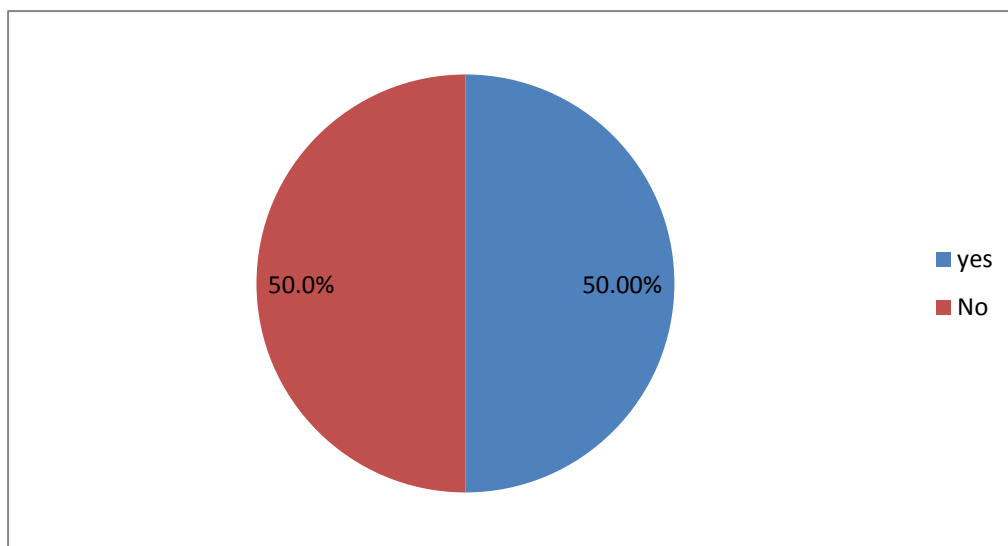
Graph-18: Side wise distribution of subjects in control and study groups



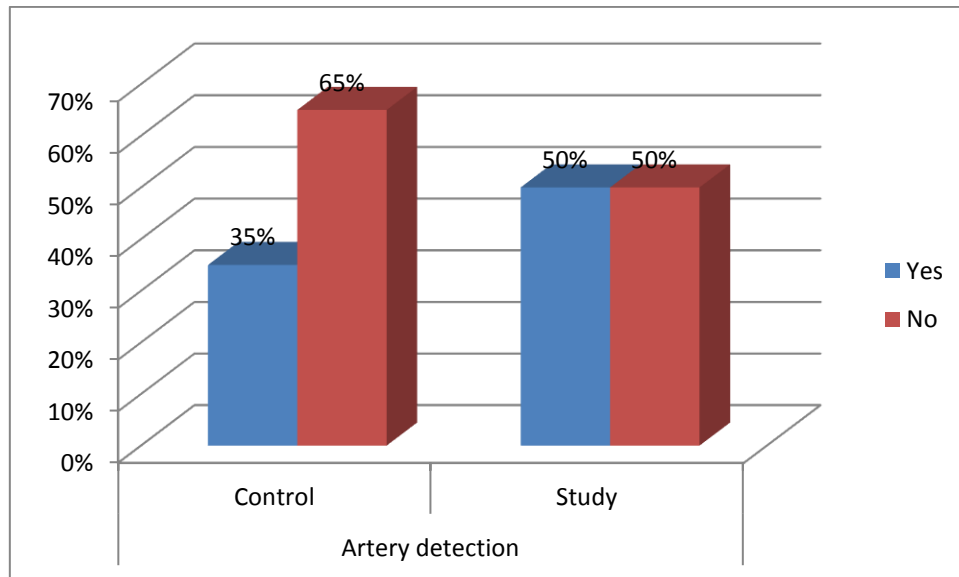
Graph-19: Detection of branch of transverse facial artery in control group



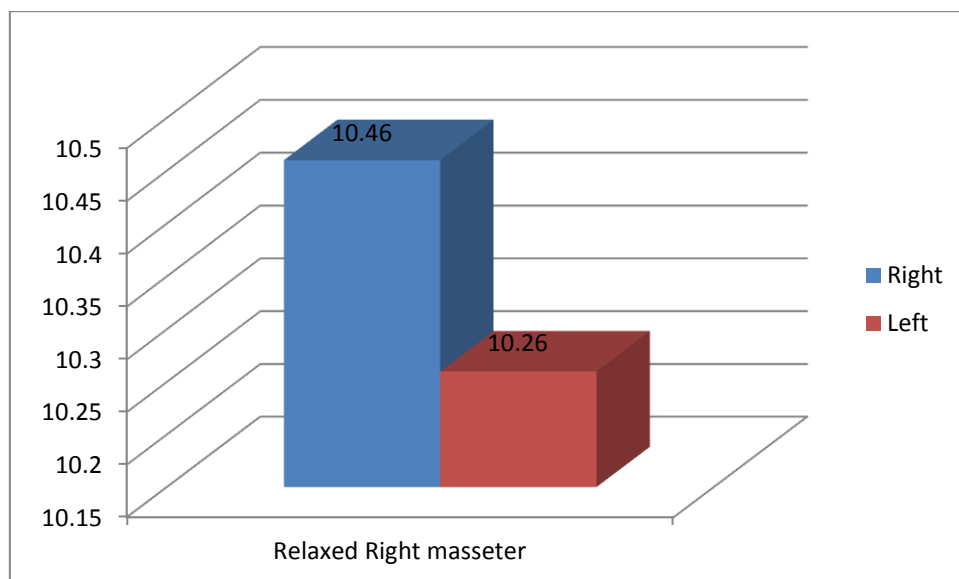
Graph-20: Detection of branch of transverse facial artery in study group



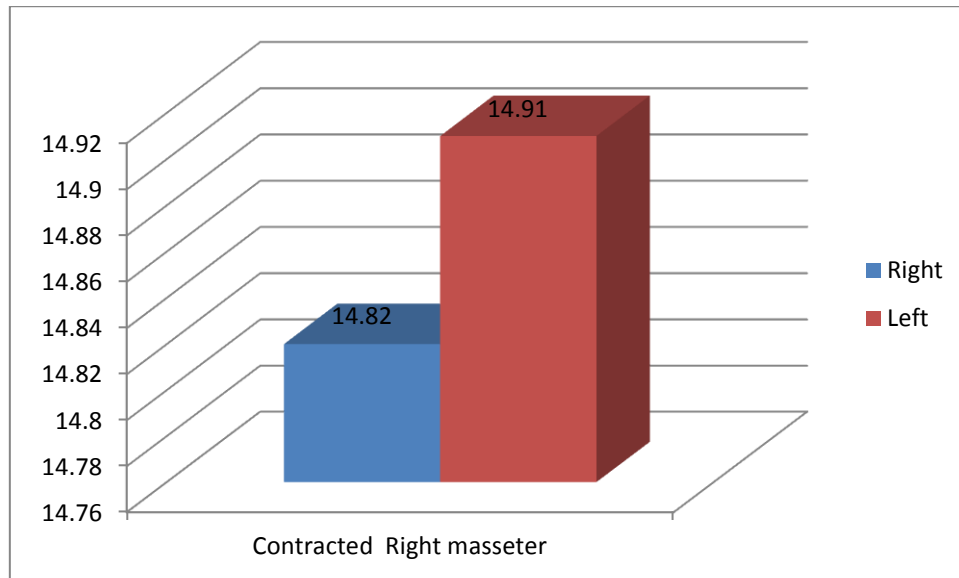
Graph-21: Detection of branch of transverse facial artery in control and study groups



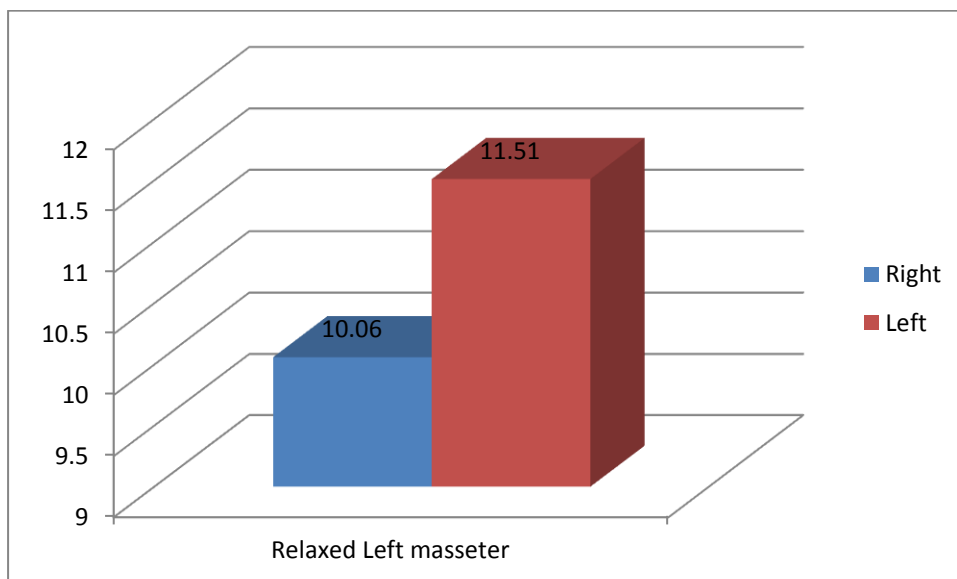
Graph-22: Correlation between relaxed right masseter measurement and side used in study group



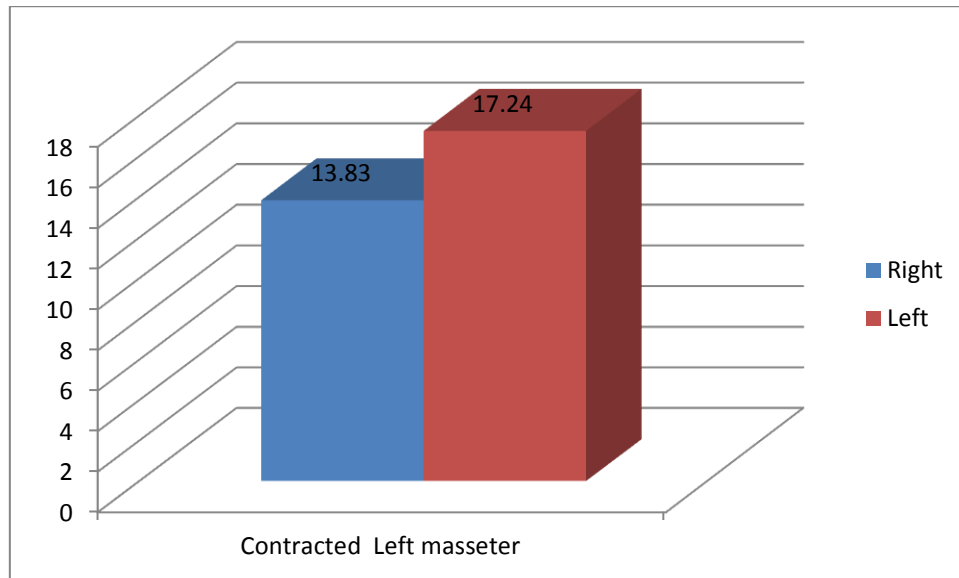
Graph-23: Correlation between contracted right masseter measurement and side used in study group



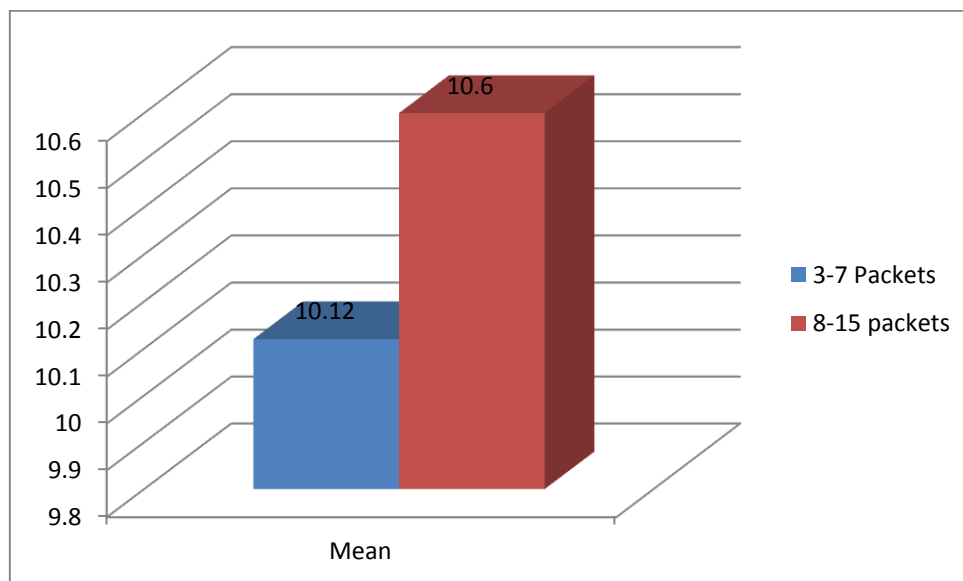
Graph-24: Correlation between relaxed left masseter measurement and side used in study group



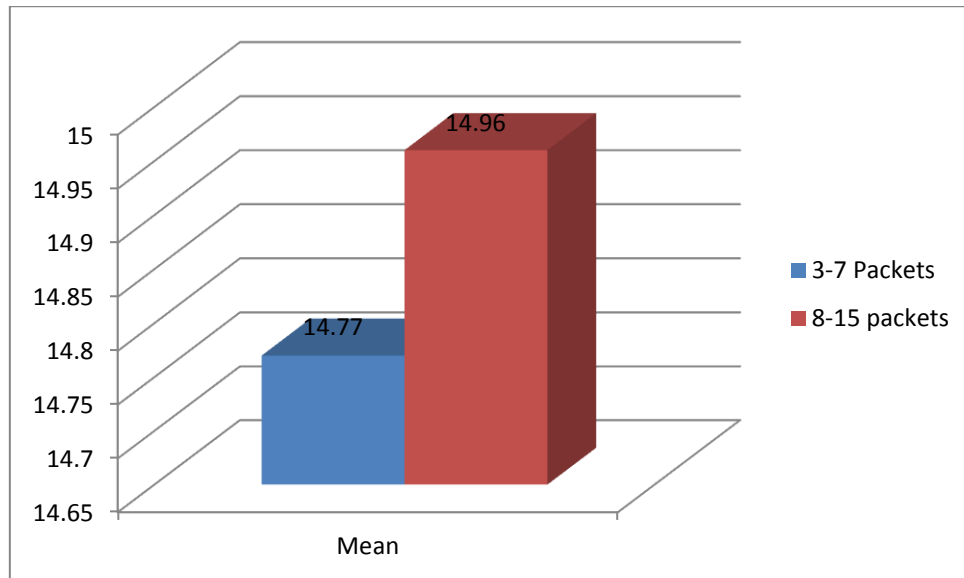
Graph-25: Correlation between contracted left masseter measurement and side used in study group



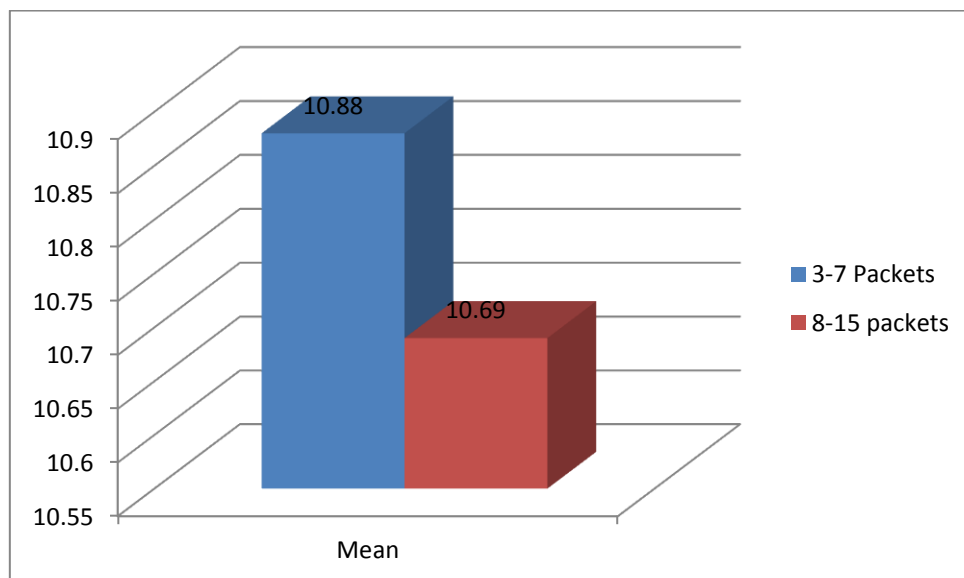
Graph-26: Correlation between relaxed right masseter measurement and number of packets of tobacco consumed per day in study group



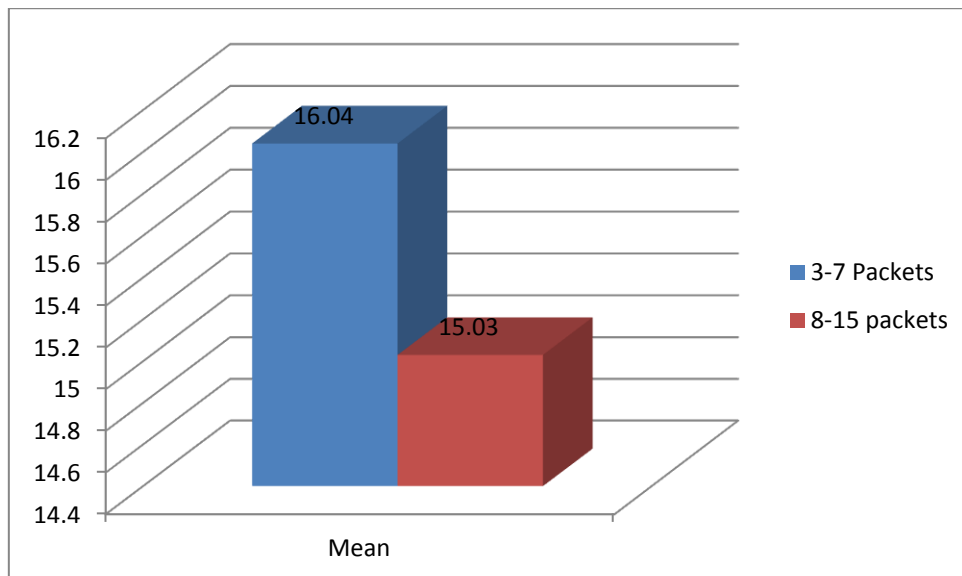
Graph-27: Correlation between contracted right masseter measurement and number of packets of tobacco consumed per day in study group



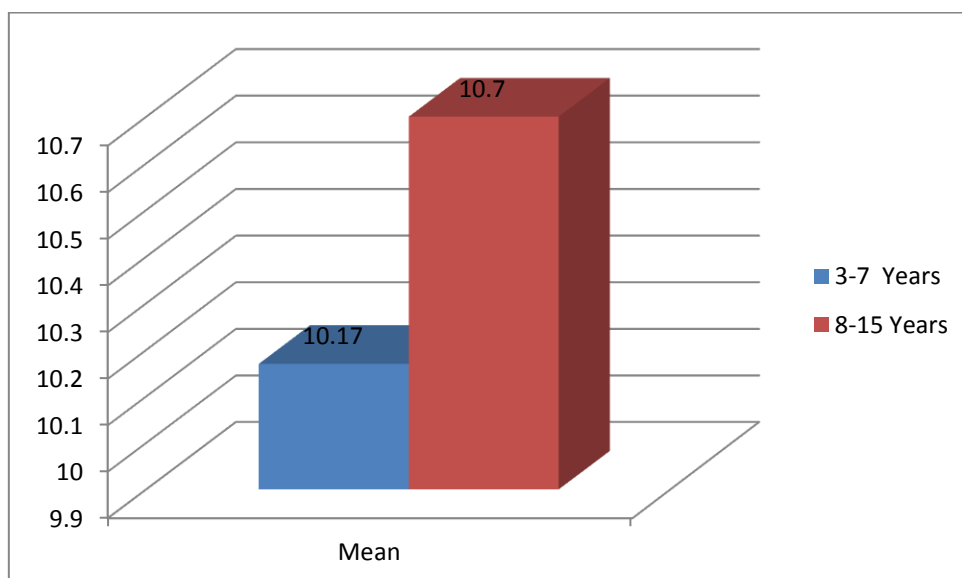
Graph-28: Correlation between relaxed left masseter measurement and number of packets of tobacco consumed per day in study group



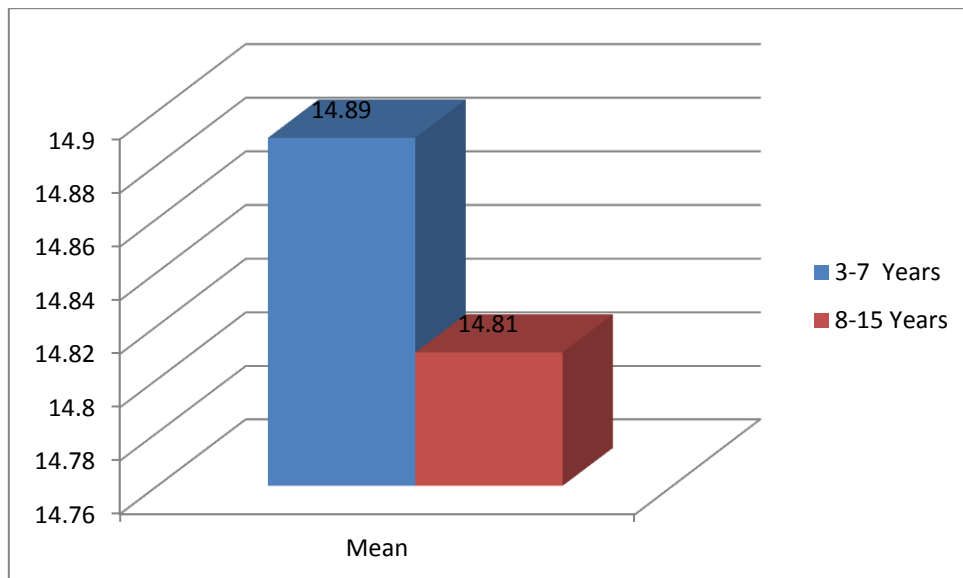
Graph-29: Correlation between contracted left masseter measurement and number of packets of tobacco consumed per day in study group



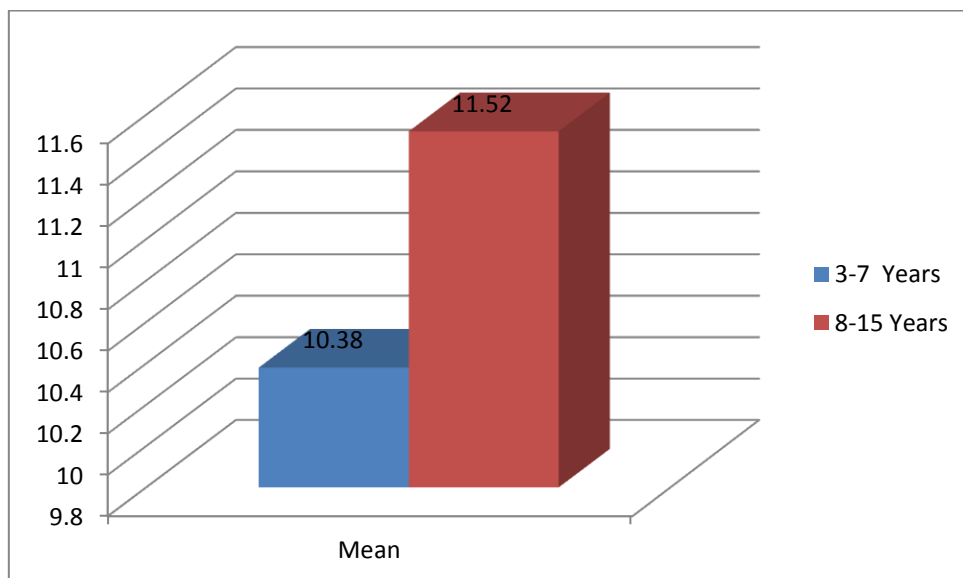
Graph-30: Correlation between relaxed right masseter measurement and number of years of chewing in study group



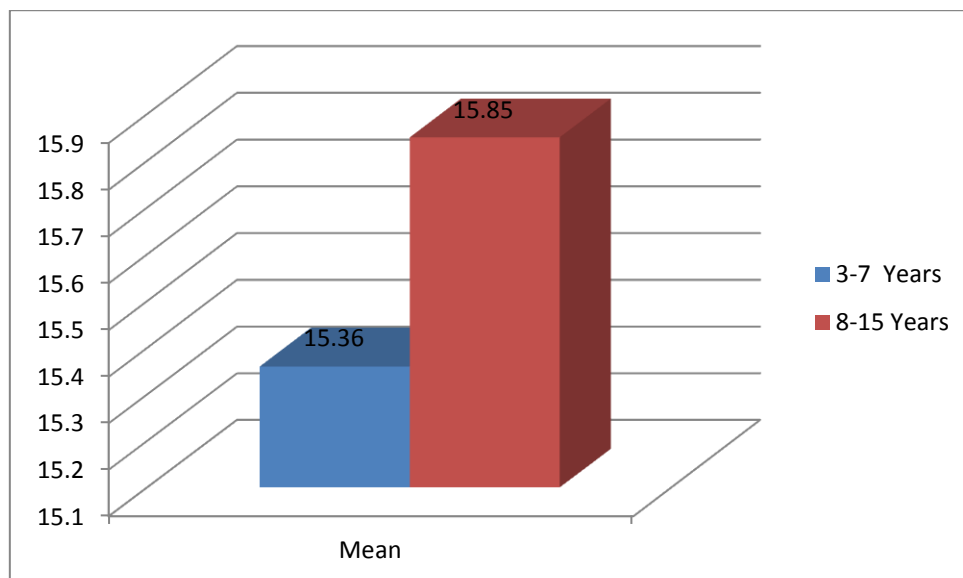
Graph-31: Correlation between contracted right masseter measurement and number of years of chewing in study group



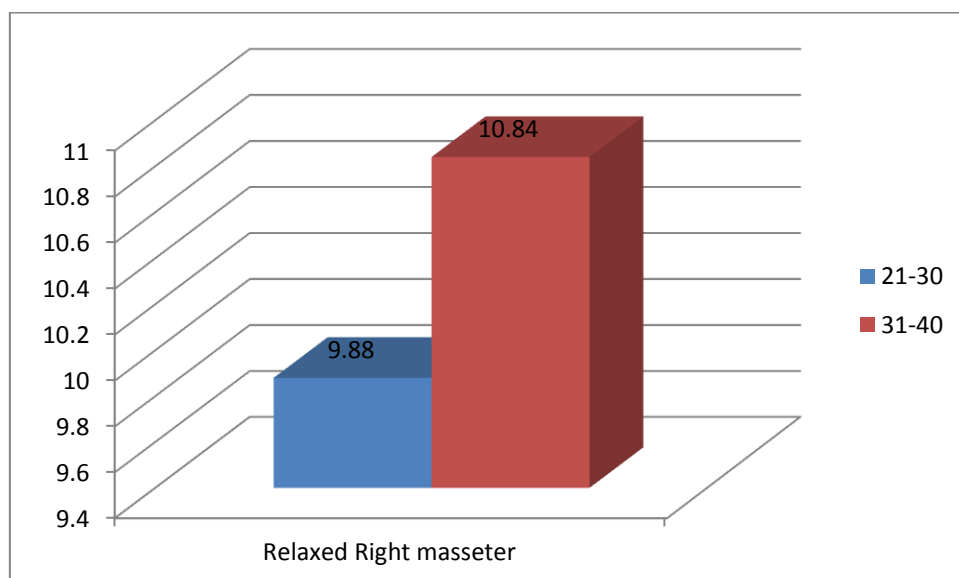
Graph-32: Correlation between relaxed left masseter measurement and number of years of chewing in study group



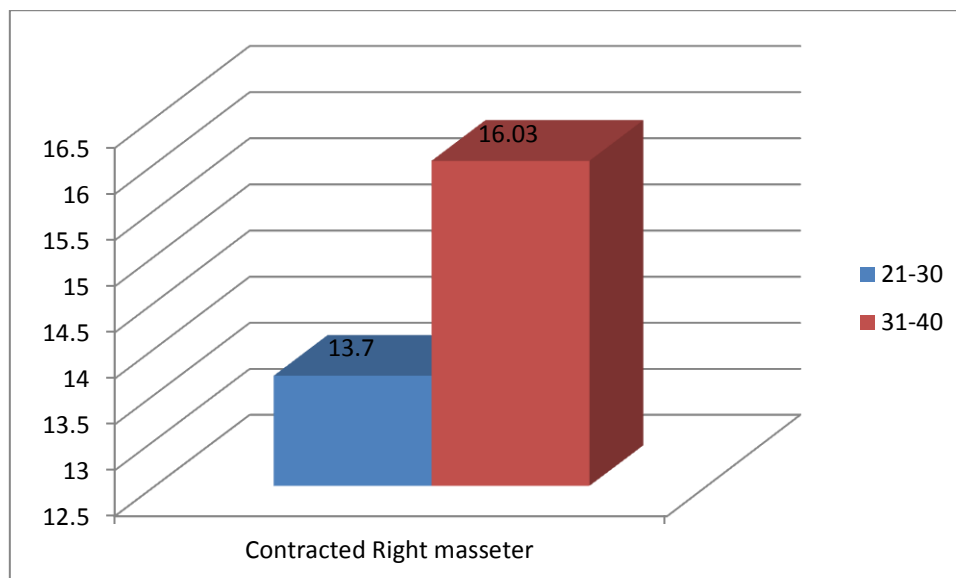
Graph-33: Correlation between contracted left masseter measurement and number of years of chewing in study group



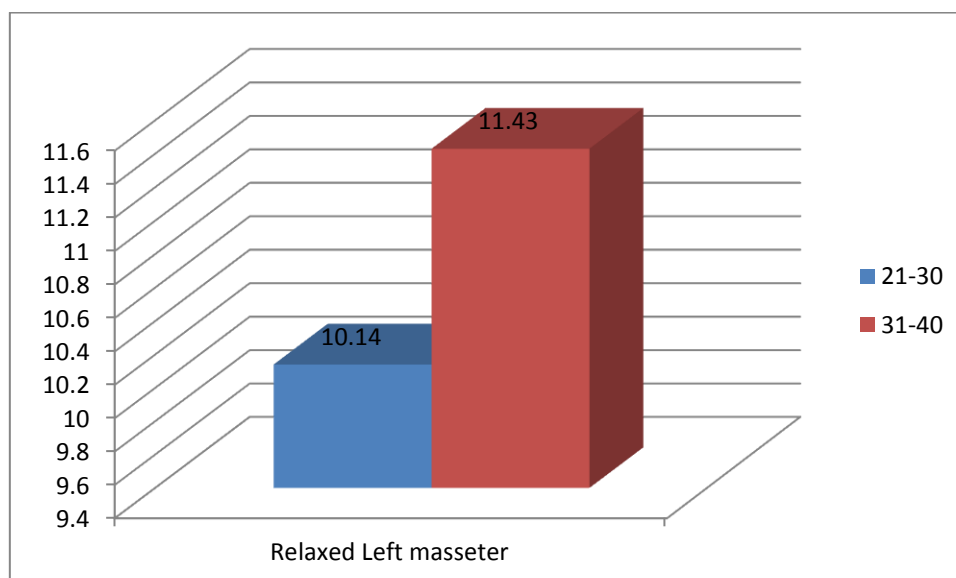
Graph-34: Correlation between relaxed right masseter measurement and age in study group



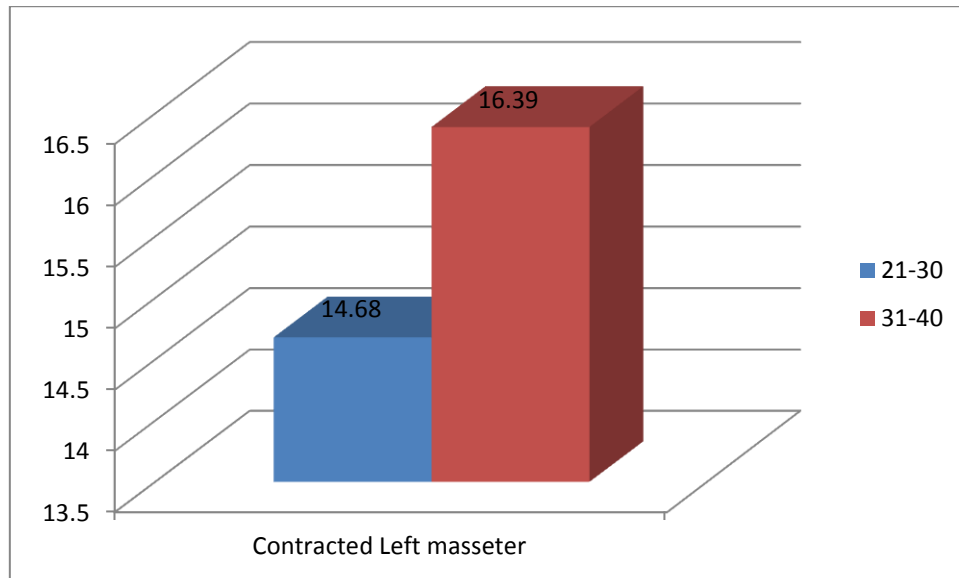
Graph-35: Correlation between contracted right masseter measurement and age in study group



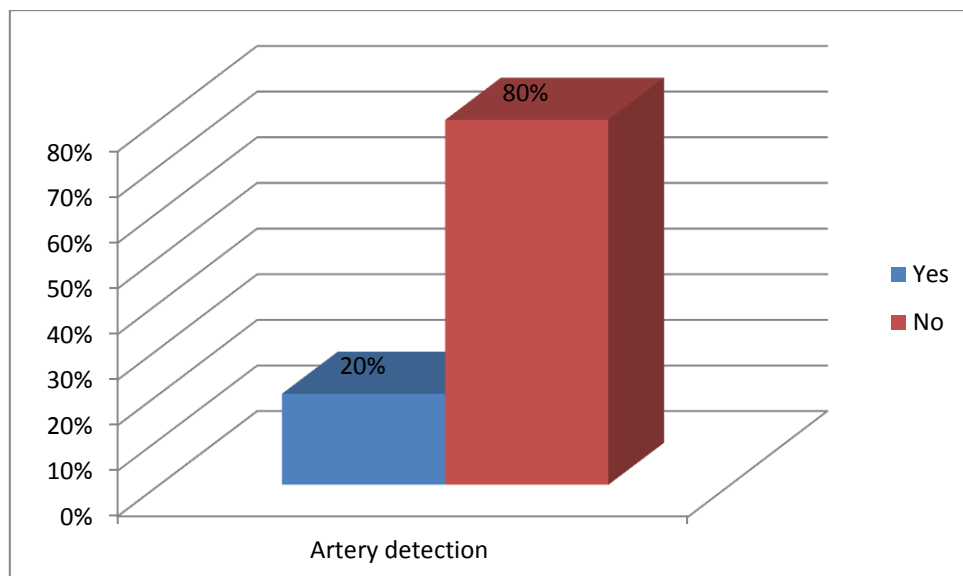
Graph-36: Correlation between relaxed left masseter measurement and age in study group



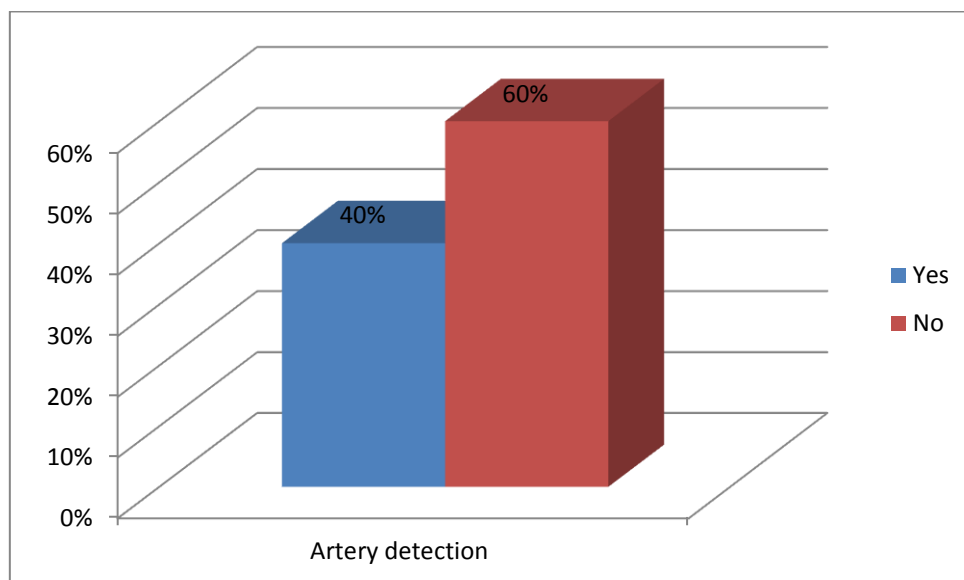
Graph-37: Correlation between contracted left masseter measurement and age in study group



Graph-38: Correlation between right side chewing and artery detection in study group



Graph-39: Correlation between left side chewing and artery detection in study group



Tobacco chewing is a popular, socially accepted, ancient custom and the introduction of commercially available tobacco products in attractive packets reinforced this practice in India. The carcinogenic effect of betel-quid and pan masala has lead to one of the highest incidence and mortality rates of oral cancer with an incidence of 83,000 incidence cases and 46,000 deaths annually in India.² The Masseter muscle is essential for mastication and play an important part in contributing to dental and articular forces which on frequent chewing of hard substances like tobacco result in overdevelopment of muscles of mastication often manifested as Muscle hypertrophy. Most research on muscle hypertrophy has concentrated on the responses of muscle cells to mechanical loading however, a number of studies also suggest that inflammatory cells may influence muscle hypertrophy.²⁵

Ultrasonography is recognized as an accurate method of measuring the thickness of masseter muscle. Studies show that direct USG measurements of masseter muscle should replace computed tomography as an definite investigation procedure.³⁶

The aim of this study is to assess changes in Masseter muscle using Ultrasonograph equipped with 11 M Hz linear array transducer in patients with the habit of tobacco chewing for more than 2 years with a frequency of more than 2 packets per day against subjects with no chewing habit.

The study was conducted between March 2012-June 2012 by Department of Oral Medicine, Diagnosis and Radiology at Ragas Dental College and Hospital and Bharat Scans Private limited, Chennai.

A case control study was conducted in 40 patients. The study sample was divided into 2 groups out of which 20 subjects were Tobacco chewers and 20 were non chewers.

Participants with mucosal lesions, Pulpoperidontal pathology, Masseter muscle pathology, TMJ disorder, Malocclusion, and females were excluded from the study.

According to the most recent Government of India's National Sample Survey data, there are 184 million tobacco consumers in India.⁹ About 40% of them are tobacco chewers. In our study among the 40 male subjects, where 20 of them are non chewers the minimum age studied was 21 years and maximum age was 40 years and the mean age obtained was 28.5 years and where 20 of them are tobacco chewers, the minimum age studied was 21 yrs. and maximum was 40 yrs. and the mean age obtained was 32 years. Our study group mean age is in accordance with **Rani, Bonu, Jha et al**¹³ whose study revealed the prevalence of tobacco chewing in age group of older than 15 years with a male predominance. Our study is also in accordance with **Rooban et al**¹⁴ who studied prevalence in only male subjects similar to our study where he found that 34 % of the population 15 years or older used chewable smokeless tobacco. **Sreeramareddy et al**¹⁵ found a 38% prevalence of

tobacco chewing among male versus 5% among women which is in accordance with our gender selection criteria where men were chosen for our study. Comparison of age groups between our control and study groups shows insignificant p value of > 0.05 due to a smaller sample size.

Muscle thickness has been considered as an indicator of jaw muscle function. The significance of chronic chewing with regard to Muscle Hypertrophy has been proven by many clinical and experimental studies.^{36,38,54}

In our routine practice we come across patients who are tobacco chewers over a period of time and not many chronic chewers present with clinically visible Masseter Muscle hypertrophy, hence we designed a study sample who did not report any signs of detectable muscle pathology. In our study we took chewing as only criteria to detect any underlying pathological changes in the muscle which is otherwise clinically normal by measuring muscle length and detecting branch of transverse facial artery by Ultrasonography and colour Doppler ultrasonography respectively. Similar comparison of Ultrasonography measurements of right and left sides in study and control group in relaxed and contracted state taking chewing as the main criteria has probably not been reported in literature, similarly artery detection in tobacco chewers and non chewers has also not been reported in literature till date.

Ultrasound has been described as an accurate and reliable imaging technique for measuring the thickness and cross sectional area of the

masticatory muscles and for detecting changes in local cross-sectional dimensions of the head and neck muscles in vivo.^{37,38,41,47}

The current study involved Subjects with Tobacco chewing habit and healthy control group with no chewing habit. Under Relaxed conditions mean thickness of right masseter muscle in our Control group was 8.7 (+/- 1.32) mm and under contracted conditions 12.49 (+/- 1.53) mm with a highly significant p value of < 0.001. This is in accordance with **Morse MH et al.**³⁶ who measured 62 masseter muscles on both sides using ultrasonography and derived the normal range to be 8.5 and 13.5 mm for relaxed and contracted state respectively. Our control group values are also in accordance with study conducted by **Rajeswari et al**⁵⁴ who studied ultrasonographic measurements of masseter muscle thickness for 40 subjects comprising of 20 controls and 20 oral submucous fibrosis patients with Masseter hypertrophy who obtained mean thickness of right relaxed and contracted muscle values of 7.66 (+/-0.87) mm and 9.98 (+/- 0.87) mm respectively in their control group. Similarly Relaxed and contracted mean values obtained for control group on left side in our study were 8.94 (+/-1.5) mm and 12.41 (+/- 1.5) mm respectively with a highly significant p value of < 0.001 which are in accordance with the study by **Morse MH et al**³⁶. Our control group left side values are also in accordance with **Rajeswari et al**⁵⁴ who obtained mean thickness of left relaxed and contracted values of 7.84 (+/-0.71) mm and 10.33 (+/- 0.51) mm respectively in their study. Our control group values are not in accordance with Contracted

values obtained in a study by **Killiardis S, Kalebo**³⁷ who obtained a range of 15.1 (+/- 1.9) mm. This variation may be due to differences in population studied.

The comparison of Right and Left Relaxed and Contracted values in Control group was not significant with a p value of > 0.05 shows that there is no asymmetry in size between Sides. This is in accordance with **PJ Close et al**⁴¹ who measured cross sectional images of 19 Male Masseter muscle and concluded that Males showed more symmetry of Cross sectional area than females.

Under Relaxed conditions mean thickness of right masseter muscle in our Study group was 10.36 (+/- 1.62) mm and under contracted conditions 14.86 (+/- 1.86) mm with a highly significant p value of < 0.001 . This is in accordance with **Rajeswari et al.**⁵⁴ who studied 20 oral submucous fibrosis patients with Masseter hypertrophy using ultrasonography and derived the range to be 9.42 (+/-1.24) mm and 13.51 (+/- 1.86) mm for right relaxed and contracted state respectively. Similarly Relaxed and contracted mean values obtained for left side in our study group were 10.78 (+/-1.53) mm and 15.53 (+/- 2.45) mm with a highly significant p value of < 0.001 respectively which is in accordance with the left side values of 9.55 (+/- 1.16) mm and 12.99 (+/- 1.75) mm in relaxed and contracted state respectively obtained by **Rajeswari et al**⁵⁴ with a significant p value of < 0.001 . The comparison of Right and Left Relaxed and Contracted values in study group was not

significant with a p value of > 0.05 shows that there is no asymmetry in size between sides. This is in accordance with **PJ Close et al⁴¹** who measured cross sectional images of 19 Male Masseter muscle and concluded that Males showed more symmetry of Cross sectional area than females.

In our Study, the mean Relaxed right masseter measurements in control and study groups obtained were 8.7 (± 1.32) mm and 10.36 (± 1.62) mm respectively and mean contracted right masseter measurements in control and study groups obtained were 12.49 (± 1.53) mm and 14.86 (± 1.86) mm respectively with a highly significant p value of < 0.001 . This is in accordance with **Rajeswari et al⁵⁴** whose mean relaxed right masseter values in control and study groups were 7.66 (± 0.87) mm and 9.42 (± 1.24) mm and contracted right masseter values in control and study groups were 9.98 (± 0.87) mm and 13.51 (± 1.86) mm respectively with a highly significant p value of < 0.001 . Similarly the mean relaxed left masseter measurements obtained in our control and study groups were 8.94 (± 1.52) mm and 10.78 (± 1.53) mm and the mean contracted left masseter measurements obtained in control and study groups were 12.41 (± 1.5) mm and 15.53 (± 2.45) mm respectively with a highly significant p value of < 0.001 . Our study group values are in accordance with **Rajeswari et al⁵⁴** whose mean relaxed left masseter values in control and study groups were 7.84 (± 0.71) mm and 9.55 (± 1.16) mm and contracted left masseter

values in control and study groups were 10.33 (+/- 0.51) mm and 12.99 (+/- 1.75) mm respectively with a highly significant p value of < 0.001.

Our study group values are also in accordance with **Ariji et al³⁹** who studied 32 patients with inflammatory changes in Masseter region using ultrasonography and obtained mean relaxed values of 8.6 mm in unaffected side versus 12.9 mm in affected side and these studies clearly indicate that our study group having muscle dimensions around the ranges mentioned above are prone to undergo pathological changes eventually leading to muscle pain and fatigue.

Bakke et al⁴⁴ studied effect of static and dynamic activity from changes in Masseter muscle thickness as a measure of oedema with assessment of pain and discomfort. Under relaxed conditions values obtained were 10.4 (+/- 2.5) mm and under contracted conditions values obtained were 12.2 (+/- 2.8) mm which is in accordance with hypertrophied muscle values obtained in our study group.

Rozylo- Kalinowska et al⁵⁰ studied ultrasonography of masseter muscle to determine normal range of masseter muscle dimensions and concluded that the range of dimensions of normal masseter muscle are very broad and in clinical practice it is essential to detect asymmetry of Right and left muscles, as well as enlargement of over 19 mm. In accordance with this study, in 2 subjects of study group one showed contracted right masseter measurement of 19.5 mm and one subject showed contracted left masseter

measurement of 19.2 mm which clearly signifies pathological changes in the muscle.

Among our Control group of 20 subjects 13 (65%) of them are habitual right side chewers whereas 7 (35 %) of them are habitual left side chewers with an insignificant p value of > 0.05 and among our study group of 20 subjects since there were 10 (50%) right and 10 (50%) left side chewers. Also on comparison among the control and study groups right and left side chewers an insignificant p value of > 0.05 was obtained which shows a sample size discrepancy in both control and study groups.

An insignificant correlation with a p value of > 0.05 with right relaxed and contracted muscle lengths and significant correlation with a p value of < 0.05 between left relaxed and contracted muscle lengths against predominant side of chewing in study group was obtained. This negative correlation with right side values could be due to smaller sample size in our study.

The correlations between number of packets of tobacco chewed and number of years of chewing with Relaxed and Contracted muscle lengths of both Right and left sides was insignificant with p value of > 0.05 in our study. This is not in accordance with **Rajeswari et al**⁵⁴ who concluded that masseter muscle thickness was increased as duration and frequency of habit was increased. This variation could be due to the clinical selection criteria of their study which consisted of patients with clinically visible Masseter muscle

hypertrophy. We may obtain positive correlations in our study with a bigger sample size.

An significant correlation with a p value of < 0.05 between age and contracted muscle lengths in study groups was obtained. This is in accordance with **S.Killiardis et al**⁴⁸ who concluded that masseter muscle was thicker in older individuals and not in accordance with **P.S Bhoyar et al**⁵⁶ who showed negative correlation with age and muscle lengths and variation in result could be due to type of study group chosen who are edentulous patients.

Colour Doppler sonography is useful in describing the arteries in and around the masseter muscle and has the potential of being used to depict the pathologic changes.⁵⁷

Our study included an added parameter of detecting branch of transverse facial artery in Masseter muscle which is the most superficial artery detected by Doppler Ultrasonography. We aimed at the detection of the superficially lying artery in both study and control groups which would signify blood flow changes in the muscle indicating muscle pathology. Detection rate was defined as percentage of subjects with visible artery in all subjects examined on colour Doppler images. One or two studies have been done in the past to assess the blood flow which could indicate underlying muscle pathology.

In our study comprising of control group of 20 subjects branch of transverse facial artery could be detected in 7 (35%) subjects whereas it could not be detected in 13 (65%) subjects. In study group of 20 subjects branch of transverse facial artery was detected in 10 (50%) subjects and not detected in 10 (50%) subjects. Individuals in whom artery was detected were on right, left or on both sides and in those with artery not detected it was not seen on either of the sides.

Our results are not in accordance with those obtained by **Ariji et al**⁵⁷ in his study who detected branch of transverse facial artery in 38 healthy volunteers. Their results showed a detection rate of 89.7% of subjects. The variation in results could be due to type of equipment, machine settings, sampling and demographic factors. No significant difference in artery detection between our control and study groups on comparison with an insignificant p value of > 0.05 was found.

Our results shows insignificant correlation between predominant side of chewing and side of artery detection with an insignificant p value of > 0.05 in our study. Hence we recommend that future studies should establish standardised scanning parameters with larger sample size. The branch of transverse facial artery not being detected in some of our study and control groups may be due to other variations like anatomical position, morphologic condition and hemodynamics.

Studies have shown that other branches such as masseteric artery and branch from external carotid artery which run from posterior part of the muscle could not be detected most of the time because the branches showed anatomically wide variations.⁵⁷ Hence we recommend that evaluation of detection rate of artery alone could not be taken as an indices to evaluate the blood flow in tobacco chewers that could signify inflammatory changes in the muscle. Colour Doppler may give conclusive results in subjects with clinically visible muscle hypertrophy and also future studies could take a bigger sample and more number of years of chewing as a criteria against haemodynamics of muscle. More studies are needed to standardise scan settings such as focal range and depth of scanning and estimation of other vessel parameters such as flow diameter and velocity of blood flow could be determined to correlate hemodynamic changes with chewing.

Our study showed association of Masseter muscle hypertrophy with tobacco chewing. Probably, First time an attempt was made to correlate the existence of masseter muscle hypertrophy in tobacco chewers with no clinically evident muscle hypertrophy which indicates that underlying muscle changes may begin in early years of chewing that could eventually lead to muscle pathology. We detected and compared branch of transverse facial artery in masseter muscle in tobacco chewers and non chewers. Though we have obtained inconclusive results on our Doppler study our attempt would pave the way for future studies trying to discover new blood flow parameters in detecting muscle pathology.

The Present study titled **Comparison of masseter muscle changes in tobacco Chewers and non chewers by ultrasonography** was conducted between March 2012 to June 2012 by Department of Oral Medicine, Diagnosis and Radiology at Ragas Dental College and Hospital and Bharat Scans Private limited Chennai, to assess masseter muscle length as an index of muscle thickness and to compare the changes in thickness in tobacco chewers and non chewers using ultrasonography and to detect branch of transverse facial artery supplying masseter muscle using colour Doppler ultrasound.

The study group comprised of a total number of 40 patients. Out of the 40 patients, 20 were tobacco chewers and 20 were non chewers. Informed consent was taken from all subjects before including them in the study. Participants with Gum chewing habit, masseter muscle pathology, mucosal lesions, pulpoperiodontal diseases, bruxism, malocclusion, TMJ disorders were excluded from the study.

The experimental subjects were made to sit comfortably on a dental chair with halogen lamp. Relevant demographic data was collected. An Intra Oral examination was carried out. The findings were recorded in a proforma. The ultrasonographic imaging was conducted in a darkened room with the patient sitting in an upright position and the Frankfort Horizontal plane parallel to the floor. Examinations were performed using Linear 11 MHz small part Transducer connected to Voluson E8 sonograph (GE medical systems Inc., USA) and the measurements made directly on the screen at the time of

scanning, recorded in millimeters. The sonograms were performed by a single experienced sonologist. The origin, insertion and anterior border of the masseter were determined by palpation. The scanning levels were orientated parallel to the occlusal plane. The transducer was held against the cheek with light pressure and oriented perpendicular to the cortex of the underlying ramus. To be perpendicular to the ramus the transducer was tilted until the ramus was depicted on the screen as a sharp white line. Scans were made in the relaxed and contracted state on both right and left sides.

The thickness of masseter muscle was measured at a site on the skin of cheek where a line joining the lateral commissure of the mouth to the intertragic notch of the ear crossed the masseter muscle. The registrations are performed three times with a time interval between the two measurements of about 3 min and the results were obtained from the mean of the measurements. The thicknesses of the muscles were measured directly on the screen of the scanner. The images of muscle during relaxation and contraction were recorded and copied on to the disc.

The study documents the following data

- The minimum age among the subjects was 21 years and maximum age was 40 years. In control group of 20 subjects the mean age obtained was 28.5 years with a standard deviation of 6.16 and in study group of 20 subjects the mean age obtained was 32 years with a standard deviation of 5.35.

- In control group of 20 subjects the Relaxed Right Masseter measurement obtained was 8.70 mm with a standard deviation of 1.3219 and Contracted Right Masseter measurement obtained was 12.49 mm with a standard deviation of 1.5317.
- In control group of 20 subjects the Relaxed Left Masseter measurement obtained was 8.94 mm with a standard deviation of 1.5209 and Contracted Left masseter measurement obtained was 12.41 mm with a standard deviation of 1.5082.
- In study group of 20 subjects The Relaxed Right Masseter measurement obtained was 10.36 mm with a standard deviation of 1.6298 and Contracted Right Masseter measurement obtained was 14.865 mm with a standard deviation of 1.8658.
- In study group of 20 subjects The Relaxed Left Masseter measurement obtained was 10.785 mm with a standard deviation of 1.5315 and Contracted Left masseter measurement obtained was 15.535 mm with a standard deviation of 2.45.
- Comparison of Relaxed and Contracted muscle lengths within the same sides in both control and study groups was highly significant with a p value of < 0.001 .

- Comparison of Relaxed and contracted muscle lengths between right and left sides in both control and study groups was insignificant with a p value of > 0.05 .
- Comparison of right and left Relaxed and Contracted muscle lengths between control and study groups was highly significant with a p value of < 0.001 .
- Out of the 20 subjects in Control group 13 (65%) of the subjects frequently used right side for chewing food and 7 (35%) of the subjects used left side for chewing food.
- Out of the 20 subjects in Study group 10 (50%) of the subjects frequently used right side for chewing and 10 (50%) of the subjects used left side for chewing.
- Comparison of sidewise distribution of subjects in control and study groups was insignificant with a p value of > 0.05 .
- In control group of 20 subjects branch of transverse facial artery was detected in 7 (35%) subjects whereas it was not detected in 13 (65%) subjects.
- In study group of 20 subjects branch of transverse facial artery was detected in 10 (50%) subjects and not detected in 10 (50%) subjects

- An insignificant correlation between Right side chewing and right relaxed and contracted muscle lengths and significant correlation between left side chewing and left relaxed and contracted muscle lengths in study group was obtained.
- An insignificant correlation between number of packets of tobacco consumed per day, number of years of chewing and relaxed and contracted muscle lengths was obtained.
- An significant correlation between age and contracted right masseter length in study group was obtained whereas insignificant correlation between age and relaxed right and left, contracted left muscle lengths were obtained.
- Insignificant correlation between predominant side of chewing and side of artery detection in study group was obtained.

To conclude our study shows significant difference between relaxed and contracted muscle lengths between our control and study groups which shows muscle undergoing hypertrophy in study group. No significant difference in artery detection between control and study groups was obtained and hence we recommend that evaluation of detection rate of artery alone could not be taken as an index of blood flow to evaluate the hemodynamics in tobacco chewers that could signify inflammatory changes in the muscle.

Many Studies have not been carried out in the past to measure masseter muscle lengths in Tobacco chewers and non chewers in spite of high prevalence of tobacco chewing in our country. Our attempt to detect and compare branch of transverse facial artery which is the most superficial artery of the masseter muscle in our control and study groups which could give us a clue on inflammatory changes in the muscle has shown inconclusive results.

The need of the hour is larger studies that need to be performed among different populations with larger sample size to establish muscle length values and hemodynamic parameters which could predict muscle pathology in tobacco chewers before arriving at a definite conclusion.

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Master Chart

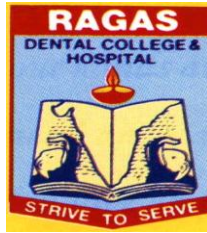
CONTROL GROUP

				1-RIGHT	RT Relaxed	RT Contracted	LT Relaxed	LT Contracted	Artery detected	Artery detected
				2-LEFT						
				Side frequently used- food chewing					Right Masseter	Left Masseter
1	Mr.Bhagvan Das	40	M	1	9	13.1	9.8	13.3	0	0
2	Mr.Karunanidhi	33	M	2	10.4	13.2	11.2	13.6	0	0
3	Mr.Paulraj	26	M	1	10	14.5	7.6	11.7	0	0
4	Malligarjun	21	M	1	8.9	12.8	9.4	14.7	0	0
5	Mr.Mani	22	M	2	7.4	13	10.6	13.3	1	0
6	Mr.Natish	21	M	1	9.7	14	9.5	14.3	0	0
7	Mr Durai	31	M	1	7.6	11	7.9	11.3	1	1
8	Mr.Subash	26	M	2	9	11	8.7	11.6	1	0
9	Mr.Nithin	25	M	1	8.7	13.8	10.8	14.1	0	0
10	Mr.Kamartheen	22	M	2	10.3	13.8	10.2	14	0	0
11	Mr Devraj	22	M	1	6.8	10.3	6.1	10.3	0	0
12	Mr.Sathish	23	M	2	10	13.6	10.4	13.6	1	0
13	Mr Suresh	23	M	2	6.4	9.9	7.2	10.2	1	1
14	Mr.Thangaduai	30	M	1	9.9	13.6	10.2	11.6	1	1
15	Mr.Mohan	32	M	2	6.8	8.9	6.6	10.6	1	1
16	Mr Ravi	34	M	1	10.5	13	8.6	11.6	0	0
17	Mr Prashanth	30	M	1	7.7	11.6	9.1	12.8	0	1
18	Mr.Rajkumar	34	M	1	8	12.5	6.5	9.9	0	0
19	Mr.Ezhumalai	35	M	1	7.6	12.6	8.8	12	0	0
20	Mr.Etiappan	39	M	1	9.3	13.6	9.6	13.7	0	0
							1-YES	0-NO		

Master Chart

STUDY GROUP

SI No.	Name	Age	Sex	No.of Packets/ day	No. of yrs.	Side used	Relaxed Right Masseter	Contracted Right Masseter	Relaxed Left Masseter	Contracted Left Masseter	Artery detected	Artery detected
							Average	Average	Average	Average	Right Masseter	Left Masseter
1	Mr.Ramesh K	25	M	10	3.5	1	9.3	13.1	8.2	11.4	1	1
2	Mr.Arunagiri	29	M	5	10	1	9.9	13.3	8.9	13	0	0
3	Mr Perumal	30	M	4	15	2	11.7	13.9	13	16.1	1	1
4	Mr.Karthik	26	M	10	4	1	8.9	12.9	9.7	12.8	0	0
5	Mr.Venkatesh	29	M	10	13	2	8.4	11.4	9.3	13.5	0	0
6	Mr.Chokalingam	28	M	10	5	2	10.7	15.4	11.8	16.6	0	0
7	Mr.Manoharan	40	M	10	13	2	10	17	13	19.2	1	1
8	Mr Parthiban	33	M	4	4	2	9.1	15.7	11.2	17.4	1	0
9	Mr.Santhoshkumar	29	M	5	6	2	9.5	14.4	9.4	16.7	1	1
10	Mr.Murugan	38	M	7	10	2	10.4	17	13.4	16.9	0	0
11	Mr.Murali	34	M	15	4	1	14.9	19.5	10	13.6	0	0
12	Mr.Ramesh V	30	M	5	10	1	10.9	14.1	10.3	12.6	0	1
13	Mr.Sathya	33	M	14	15	2	13.6	17	12.8	17.4	0	0
14	Mr.Karthik	40	M	6	5	1	8.9	15	9.2	15.3	0	0
15	Mr.Sudheer	40	M	5	5	1	11.6	16.3	11.3	15.4	0	0
16	Mr.Arulkumar	35	M	5	6	1	9.5	14.7	12	15.2	0	1
17	Mr.Ramesh	40	M	9	4	2	9.5	14	11.1	16.8	1	1
18	Mr.Ezhumalai K	25	M	15	5	1	11.3	15.5	11.3	15.9	0	1
19	Mr.Venkatesan	26	M	8	5	1	9.4	13.8	9.7	13.1	1	0
20	Mr.Kumar	28	M	4	4	2	9.7	13.3	10.1	15.2	0	0



RAGAS DENTAL COLLEGE AND HOSPITAL

Department Of Oral Medicine, Diagnosis and Radiology

CASE SHEET PROFOMA

Comparison of masseter muscle changes in tobacco chewers and non- chewers using ultrasonography

A] General Information

DATE: S.NO. OP. NO.

1. Name:

2. Age:

3. Sex: a. Male b. Female

4. Occupation:

- a. Unemployed
- b. Professional
- c. Administration
- d. Trade/Business
- e. Student

5. Address:

6. Income:

a. <Rs.1000/month b. > Rs.1000-5000/month c.>Rs.5000 /month

B] Past Dental History:

C] Habit

Tobacco chewing: a) Type
 b) Duration
 c) Frequency

D] Extra oral Examination

TMJ Examination

Mouth opening

Closing

Protrusion

Retrusion

Lateral Movements

On Palpation

Tenderness

Crepitus

Clicking

Examination of Muscles of mastication

Tenderness : Present/Absent

E] Intra oral Examination

Decay:

Missing:

Filled:

Tobacco stains:

Attrition:

Occlusion:

F] Investigations

Ultrasonogram

Doppler ultrasound

G] Results

Right side Masseter muscle

Measurements on Relaxation

Measurements on Contraction

Left side masseter muscle

Measurements on Relaxation

Measurements on Contraction

Detection of branch of transverse facial artery

Detected

Not detected

CONSENT LETTER

I _____, the under signed hereby give my consent for the performance of undergoing ultrasound Examination of Masseter muscle to assess and compare the changes produced by chewing tobacco among chewers and non chewers conducted by Dr. Priya R, under the guidance of Dr.S. Kailasam MDS, Professor and Head of Department of Oral medicine Diagnosis and Radiology, Ragas dental college and Hospital. Chennai. I have been informed and explained about the evaluation procedure, risks involved and likelihood of successes. I also understand and accept this as part of study protocol, thereby voluntarily, unconditionally freely give my consent without any fear or pressure in mentally sound, conscious state to participate in the study.

Witness/Representative

Patient Signature

(if any)

Date:

ஒப்புதல் படிவம்

----- என்கின்ற நான், சென்னை ராகாஸ் பல் மருத்துவக் கல்லூரி மற்றும் மருத்துவமனையின் வாய் மருத்துவம் மற்றும் ஊடுகதிர் துறையில் பேராசிரியர் மரு. S கைலாஸ் (M.D.S) அவர்களின் மேற்பார்வையில், முதுநிலை (M.D.S) பட்டப்படிப்பு பயிலும் மரு. பிரியா. ரா அவர்கள் மேற்கொள்ளும் “புகையிலை மெல்லும் பழக்கத்தினால் கன்னத்தசையில் ஏற்படும் மறுபாடுகளை அல்ட்ராசோனோகிராபி முறைமூலம் புகையிலை உபயோகிப்போர் மற்றும் புகையிலை பழக்கம் இல்லாதோரிடம் ஒப்பிட்டு கண்டறியும் ஆய்வு” என்கின்ற ஆராய்ச்சிக்கான பரிசோதனைக்கு என்னை உட்படுத்துவதற்கு எனது மனமுவந்த பரிபூரண சம்மதத்தினை அளிக்கிறேன்.

மேலும் எனக்கு என்னுடைய நோயின் தன்மையைபற்றியும், அதனால் ஏற்படக்கூடிய விளைவுகளைப்பற்றியும் எடுத்துக் கூறப்பட்டுள்ளது எனவும், இந்த பரிசோதனைக்கு நான் எந்தவித அச்சமுமின்றி தன்னிச்சையாகவும், தெளிவான முழு மனதுடன் என்னுடைய பரிபூரண சம்மதத்தினை அளிக்கிறேன் என இதன் மூலம் தெரியப்படுத்துகிறேன்.

சாட்சியாளர்கள் :

இப்படிக்கு